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RESEARCH IN BIOMEDICAL, HEALTHCARE AND PHARMACEUTICAL SCIENCE

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PREFACE

Welcome to the dynamic world of biomedical, healthcare, and pharmaceutical research, where the pursuit of knowledge intersects with the relentless quest for advancements that impact human health and well-being. This volume, "Research in Biomedical, Healthcare, and Pharmaceutical Science," is a compilation of cutting-edge research contributions that reflect the diversity and depth of inquiry in these critical fields.

In the rapidly evolving landscape of healthcare and life sciences, researchers and practitioners alike are confronted with a myriad of challenges and opportunities. This book serves as a testament to the collective efforts of scholars, scientists, and professionals who have devoted their expertise to unraveling the complexities of biological systems, developing innovative medical technologies, and advancing pharmaceutical interventions.

The scope of this book spans a wide spectrum of topics, encompassing fundamental research, applied sciences, and translational efforts that bridge the gap between benchside discoveries and bedside applications. From molecular investigations that elucidate the intricacies of cellular processes to clinical studies that inform evidence-based medical practices, the chapters within this volume showcase the interdisciplinary nature of contemporary research in biomedical, healthcare, and pharmaceutical science.

The significance of the research presented herein extends beyond the confines of academic inquiry. The findings and innovations discussed in these pages have the potential to shape the future of healthcare delivery, influence public health policies, and inspire the next generation of scientists and practitioners. The collaborative spirit evident in these contributions reflects a shared commitment to advancing human health and fostering a deeper understanding of the biological underpinnings of disease.

May this volume serve as a source of inspiration, a reference for current and future researchers, and a catalyst for transformative advancements in the fields that hold the key to our collective well-being.

Editors

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AUTOIMMUNE PEMPHIGUS: ADVANCES IN THE THERAPEUTIC STRATEGIES AND MANAGEMENT PROTOCOL

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Introduction:

Pemphigus vulgaris (PV) is a potentially fatal autoimmune, intraepithelial disease characterized by flaccid blisters and erosions of the skin and mucous membranes and histologically by acantholysis. It is mediated by circulating desmoglein-reactive autoantibodies directed against keratinocyte cell surfaces.

The incidence of PV is 0.1–0.5 cases/100 000 people/year[1] with an equal predilection for both genders. The mean age of onset is 50–60 years. PV can be associated with other autoimmune disorders, particularly rheumatoid arthritis and lupus erythematosus. The disease is characterised by the loss of cellular adhesion due to the formation of IgG autoantibodies against desmoglein 1 and/or 3. (1) Types 1 and 3 desmogleins are found within skin whereas mucosa contains only the type 3 variant. The severity and site of the disease will depend on whether types 1 and 3 desmogleins are affected. Patients with high-serum anti-desmoglein 3 antibodies but low-anti-desmoglein 1 antibody will have only mucosal involvement. This is because the function of desmoglein 1 is preserved preventing the development of skin lesions.

Loss of cell-to-cell adhesion results in the development of intraepithelial blisters that rupture following minimal trauma, leaving behind painful sloughing eroded areas of skin and/or mucosa. Diagnosis requires biopsy of the peri-lesional tissue for histopathology to show acantholysis and direct immunofluorescence to demonstrate the presence of IgG antibody along the cell surface within the intercellular space.

PV is characterized by diversity that makes every patient a unique challenge. Patients might present with lesions solely on mucous membranes and have limited cutaneous or extensive mucocutaneous involvement. Comorbidities such as diabetes mellitus, hypertension, previous or existent malignancies, chronic infections, and associated complications might limit available treatment options. There are many therapeutic interventions in use that have not been evaluated in well-designed randomized controlled trials (RCTs). Sample sizes of conducted trials are often small and have been occasionally evaluated as insufficient to yield definitive results. Most published data regard patients during disease onset.

The primary objective of the therapeutic management of PV is initially to control the disease, heal the bullous skin and mucous lesions, and minimize the associated functional impairment. Subsequently, the real challenge is to prevent relapses in the long run and avoid adverse events associated with the prolonged use of steroids and immunosuppressive agents. Such intent requires close clinical monitoring of efficacy and safety of treatment.

Oral lesions are challenging, since their response to treatment is much slower in comparison to cutaneous lesions. The aim of pharmacologic therapy for PV is to reduce inflammatory response and autoantibody production. The exact mechanism of immunosuppressive medications in pemphigus is unknown but it is believed that these therapies act by inhibiting B cell and autoantibody production which contribute to disease activity. Adding an adjuvant agent is proven to lower the risk of relapse. However, this effect is lost when comparing specific adjuvant medications. Also, adjuvant therapy does not improve remission rates, time to disease control, time to relapse, or the incidence of death in pemphigus.

First line of therapy:

Corticosteroids: The anti-inflammatory effects are mediated either by direct binding of the glucocorticoid/glucocorticoid receptor complex to glucocorticoid responsive elements in the promoter region of genes, or by an interaction of this complex with other transcription factors, in particular activating protein-1 or nuclear factor-kappa B. [(2)] Glucocorticoids inhibit many inflammation-associated molecules such as cytokines, chemokines, arachidonic acid metabolites, and adhesion molecules. In contrast, anti-inflammatory mediators often are up-regulated by glucocorticoids. [(3)]

Topical corticosteroids:

Mild localized lesions of oral mucous membrane pemphigus in patients with low titers of circulating auto antibodies may be controlled, at least temporarily, with ***topical corticosteroid rinses or creams***, including agents such as clobetasol propionate. Suspensions and mouthwashes are also effective for mild lesions. Systemic CSs are still the first-line treatment for PV. One of the main concerns in uncomplicated patients is when rapid control of the disease is achieved by monotherapy with CSs. Control of disease activity is usually achieved within several weeks. Complete remission on minimal treatment needs months, while complete remission off treatment often requires several months or even years of therapy. A second debate often concerns whether to start with a low or high dose of CSs.

The guidelines by EDF and European Academy of Dermatology and Venereology recommend initial prednisolone dose at 0.5 mg–1.5 mg/kg/d and if control of the disease is not reached within 2 weeks, a higher prednisolone dose (up to 2 mg/kg) could be administered.

The optimal dose has not been validated. A controlled trial showed no significant difference regarding the duration of remissions and relapse rates at 5 years in patients randomized to treatment with either low-dose oral prednisolone (1 mg/kg/d) or high dose oral prednisolone (2.0–2.5 mg/kg/d).

Once remission is induced and maintained with healing of the majority of lesions, the dose can be tapered by 25%. Reduction may be performed biweekly with slower decreases when doses below 20 mg/d are reached. CSs can be combined with an immunosuppressive agent, particularly when complications due to expected prolonged use (4 months) such as hypertension, diabetes mellitus, and osteoporosis are expected. Even though the superiority of steroids plus adjuvant therapy over prednisolone monotherapy is debatable, considerable research effort has been directed at finding the optimal steroid-sparing agent. [(4)]

A systematic review that evaluated RCTs with adjuvant therapy with azathioprine, mycophenolate mofetil (MMF), cyclophosphamide, cyclosporine, intravenous immunoglobulin (IVIG), plasma exchange, and infliximab in PV patients concluded that adjuvants were not beneficial for achieving remission, but were found to collectively decrease the risk of relapse by 29%.

Pulse regimen:

If doses of prednisolone above 100 mg/d are required, pulse treatment with either oral or intravenous (IV) steroids may be considered. A regimen of IV betamethasone in combination with oral prednisolone in PV patients showed a shorter time to remission, clinical resolution (including oral lesions), and minor adverse effects when compared to monotherapy with oral prednisolone. A common pulse regimen used is 100 mg/d of IV dexamethasone for 3 days every 2–3 weeks. However, pulsed CSs do not appear to have additional benefit on top of conventional first-line treatment with oral prednisolone and immunosuppressive adjuvants. Most of the studies investigating the efficacy of pulse steroid treatment involve patients with refractory PV. In such patients, pulsed therapy with IV dexamethasone could be a reliable alternative when other options have failed. [5]

Second-line treatment:

Second-line treatment in the case of contraindications to glucocorticoids or complications due to expected prolonged use (4 months) consists in the combined or single use of immune suppressants such as azathioprine, MMF, dapsone, methotrexate, cyclophosphamide, and cyclosporine. In recent years, the use of IVIG and biologics such as infliximab, and especially rituximab, has been reported to produce excellent results in refractory cases.

1. Azathioprine:

Azathioprine is among the oldest pharmacologic immunosuppressive agents in use today. The drug is a purine analog, and the accepted mechanism of action is at the level of DNA. Both in vitro and in vivo, azathioprine is metabolized to 6-MP through reduction by glutathione and other sulphydryl-containing compounds and then enzymatically converted into 6-thiouric acid, 6-methyl-MP, and 6-thioguanine (6-TG). Ultimately, azathioprine can then become incorporated into replicating DNA and can also block the de novo pathway of purine synthesis. [6,7]

Azathioprine is one of the main adjuvants used in PV. It is considered a first-line adjuvant immunosuppressant according to the EDF guidelines. Dose varies between 1 and 3 mg/kg/d, based on the activity of the thiopurine methyltransferase (TPMT) enzyme, involved in the metabolism of the drug. When TPMT levels are high, normal doses of azathioprine (up to 2.5 mg/kg/d) are administered, while adults with PV and intermediate or low TPMT levels should receive a maintenance dose (up to 0.5–1.5 mg/kg/d). Azathioprine should not be used in patients with any TPMT activity. A dose of 50 mg/d could initially be administered, and if no idiosyncratic reactions occur, it could be increased after a week. In case of idiosyncratic reactions, it should be discontinued.

The primary benefit of adjuvant azathioprine is its steroid-sparing effect. Azathioprine has been reported to require a lower cumulative CS dose for remission, with some investigators

reporting superior steroid-sparing effect when compared to MMF and cyclophosphamide, while others concluded that cyclophosphamide is superior.

Adverse events of adjuvant azathioprine treatment are decreased when compared to steroid monotherapy without any compromise in rates of clinical remission. [8]

2. **Mycophenolate mofetil**

Mycophenolate mofetil is a prodrug of mycophenolic acid (MPA), an inhibitor of inosine monophosphate dehydrogenase (IMPDH). This is the rate-limiting enzyme in de novo synthesis of guanosine nucleotides. T- and B-lymphocytes are more dependent on this pathway than other cell types are. Moreover, MPA is a fivefold more potent inhibitor of the type II isoform of IMPDH, which is expressed in activated lymphocytes, than of the type I isoform of IMPDH, which is expressed in most cell types. MPA has therefore a more potent cytostatic effect on lymphocytes than on other cell types. This is the principal mechanism by which MPA exerts immunosuppressive effects. [9]

MMF is a safe steroid-sparing agent. It is considered a first-line adjuvant immunosuppressant according to the EDF guidelines. The optimal dose is weight dependent with a dose of 2 g/d recommended for the average patient of 75 kg. Progressive dose increases by 500 mg/wk until the final dose of 2 g/d has been proposed to avoid gastrointestinal adverse events. Efficacy is debated. In a recent RCT, MMF (2 or 3 g/d) plus oral CSs was not found to be superior when compared with oral CSs and placebo in patients with mild or moderate PV. [8] The primary end point was patients responding to treatment. Other investigators have also reported no clinical benefit using adjuvant MMF to steroids in patients with PV. MMF in combination with prednisolone seems to have a more prominent beneficial role in patients with relapses of PV or in cases of refractory PV who have failed previous treatments.

3. **Cyclophosphamide**

Cyclophosphamide is an inactive drug. With the help of cytochrome P-450 oxidase system in the liver, the inactive drug is converted into Phosphoramide mustard and acrolein which are very active compounds. Phosphoramide mustard has an ability to introduces alkyl radicals into DNA strands with interferes DNA replication by forming DNA cross-linkage. Cyclophosphamide also produces immunosuppressive effects possibly through a cytotoxic effect on lymphocytes. [10]

Cyclophosphamide is considered a second-line immunosuppressant adjuvant therapy according to the EDF guidelines. It can be administered either as a 500 mg IV infusion or as 2 mg/kg/d orally.

Cyclophosphamide monotherapy has not been able to demonstrate any benefit over prednisolone. Some authors report superiority over azathioprine or mycophenolate as adjuvant therapy. [11] The potential long-term side effects (infertility, increased risk of cancer, infections, genitourinary complications, and lymphopenia) further limit cyclophosphamide's use.

4. **Dapsone**

Dapsone has anti-inflammatory and immune modulatory effects, which are thought to come from the drug's blockade of myeloperoxidase. Dapsone is recommended in a dose of 100

mg/d or up to #1.5 mg/kg/d as a steroid-sparing agent. An RCT reported superiority of dapsone over placebo as a steroid-sparing agent when the primary end point was to taper prednisolone to #7.5 mg/d. However, dapsone did not exhibit any benefit on remission of the disease. Before initiating therapy with dapsone, serum G6PD activity should be tested. (12)

5. Methotrexate

The mechanisms of action of methotrexate are complex. Developed as a folic acid analogue, methotrexate inhibits purine and pyrimidine synthesis, which accounts for its efficacy in the therapy of cancer as well as for some of its toxicities. Recently, many studies have focused on the adenosine-mediated anti-inflammatory effects of methotrexate. (13)

Methotrexate could be used as a steroid-sparing agent in a dose of 10–20 mg/wk. Literature data assessing its efficacy in PV treatment are scarce. A recent retrospective study reported that 21 out of 25 patients downgraded PV severity and were able to taper steroids after 6 months when using adjuvant therapy with 15 mg of methotrexate per week.

6. Rituximab

Rituximab is an anti-CD20 monoclonal humanized antibody with the potential to reduce desmoglein autoantibodies and selectively deplete B cells. Anti-cancer monoclonal antibodies (mAbs) can mediate anti-tumour effects by a variety of mechanisms including signalling resulting in cell cycle arrest, direct induction of apoptosis, and sensitization to cytotoxic drugs, complement dependent cytotoxicity (CMC) and antibody dependent cellular cytotoxicity (ADCC) Rituximab is indicated in patients who remain dependent on more than 10 mg prednisolone combined with an immunosuppressive adjuvant according to the EDF. (14)

Administration schedule in literature is either 1000 mg IV every 2 weeks or 375 mg/ m^2 every week. The same dosage can be administered again in case of clinical relapses. A meta-analysis on treatment with rituximab in severe pemphigus showed remission in approximately 95% of the total patients. Prophylactic infusion after complete remission does not seem to provide any additional benefit. The incidence of serious infections was 3.9% using the weekly protocol but 15.21% in the biweekly protocol. However, the incidence of unforeseen fatal infections such as progressive multifocal leuko encephalopathy cannot be estimated due to the rarity of the disease. Concomitant long-term antibiotic and prophylaxis for herpes virus has been shown to drastically reduce the rate of infections.

The clinical benefits of ritumaxib are noticed within 2 to 3 months of infusion and can last years. One study found that the combination of rituximab for 3-weeks and IVIg for the fourth week followed by maintenance monthly rituximab and IVIg results in rapid clinical resolution, steroid cessation, and prolonged remission. Another study found similar response and relapse rates with rituximab alone. Rituximab does not eliminate the need for steroids or immunosuppressive agents, and most patients in published studies did use such therapy along with rituximab. Before initiation of treatment, physicians should have a specific goal and end point. They should also be aware of its potential side effects and lack of information on its long-term effects. Patients should be carefully monitored during and after therapy. (15)

7. Intravenous immunoglobulins

Treatment with IVIG can be used in refractory disease or in case of contraindications to immunosuppressive adjuvants. The usual dose is 2 g/kg/cycle IV administered over 2–5 consecutive days, monthly. A multi-centre RCT that compared various doses of IVIG and placebo infusion demonstrated the beneficial effect of IVIG in the management of refractory pemphigus, indicating a dose–response relationship of the treated patients. IVIG could be used as adjuvant therapy to systemic CSs and immunosuppressive adjuvants. Treatment should be performed over several days to avoid adverse effects such as headache and nausea. IVIG could induce aseptic meningitis in patients who commonly experience migraines, and is contraindicated in patients with complete IgA deficiency. (16)

8. Infliximab

Infliximab is a chimeric monoclonal antibody against tumor necrosis factor alpha (TNF-α). TNF-α has been found to be strongly expressed by the acantholytic cells in PV.49 There are several case reports and case series of PV patients successfully treated with infliximab. On the other hand, there are also several case series and a small comparative study showing no benefit in patients with PV treated with infliximab. (17)

There is also one case of a patient treated with infliximab for rheumatoid arthritis who developed pemphigus foliaceus. In the context of current evidence, infliximab does not have a role in the treatment of PV.

9. Gold:

Most studies have used intramuscular gold, initially at a dose of 50mg per week if test doses were tolerated. It was used successfully as monotherapy in five patients with an associated fall in indirect immunofluorescence titres. However, it has more commonly been used as an adjuvant drug and steroid sparing effects are reported. Gold can be considered as an alternative to more established adjuvant drugs if they cannot be used. (18)

10. Tetracycline:

Variable combinations of tetracyclines with or without nicotinamide have been described in pemphigus vulgaris. Sixteen patients were given nicotinamide 1.5g and tetracycline 2g daily. In 12 patients, no systemic steroids were given and of these only three cleared and three improved. Tetracyclines with or without nicotinamide could be considered as adjuvant treatment, perhaps in milder cases of pemphigus vulgaris (19)

11. Chlorambucil:

Seven patients with pemphigus vulgaris who had failed to respond to other steroid or immunosuppressive combinations were given oral chlorambucil 4mg/day titrated upwards according to clinical response. There was improvement or remission in five patients and a steroid sparing effect was reported. Chlorambucil could be considered as an adjuvant drug if more established options cannot be used but there is limited data to support its use.

Therapeutic plasma exchange – plasmapheresis:

Plasmapheresis is an extracorporeal blood purification technique, in which the blood is continuously removed from the patient and separated into cellular components and plasma; the

cellular compartments are returned to the patients along with replacement fluid like albumin. Plasma exchange has been described as an effective adjuvant therapy in severe PV patients in controlling disease activity by reducing serum levels of autoantibodies. Plasma exchange can be performed using a centrifugation device used in blood banks. The double filtration plasmapheresis is a newer procedure that currently prevails because of its safety advantage

In double filtration plasmapheresis, immunoglobulins are selectively removed, while the loss of albumin is minimized. There is no standardized protocol for the number and frequency of sessions; however, four or five plasma exchanges, each exchange consisting of 1–1.5 plasma volumes, over a period of 7–10 days constitute an adequate short-term therapy to remove 90% of the total initial body immunoglobulin burden. Plasma exchange is relatively safe, and the risk of infection associated with it is mainly due to the steroids and immunosuppressives given along with it. Other transient and minor adverse effects of plasma exchange that have been reported include thrombocytopenia, hypo gammaglobulinemia, fluid overload leading to hypertension and pulmonary edema, hypoproteinemia, anaemia, leukopenia, and hypocalcaemia. [(20)] Because of the rapid fluid shift occurring as a result of removal of proteins, which maintain the osmotic pressure, it can lead to severe problems in patients with compromised cardiac function.

Immunoadsorption:

Rapid removal of circulating autoantibodies against Dsg1 and Dsg3 can be achieved by immune adsorption. It is indicated in patients with refractory PV when CSs combined with azathioprine or mycophenolate fail to control the disease. Four treatments of immune adsorption on 4 consecutive days (2.5-fold plasma volume/d), repeated after 4 weeks, if needed, are the recommended schedule. Treatment could be undertaken in combination with immunosuppressive agents such as rituximab and cyclophosphamide.

Contraindications include severe systemic infections, cardiovascular diseases, and haemorrhagic diathesis. While immune adsorption is far superior to plasmapheresis in terms of efficacy and safety, the high cost of the adsorbers is the major limiting factor. [(21)]

Extracorporeal photochemotherapy:

Extracorporeal photo chemotherapy involves the collection of mononuclear cells with a cell separator, their irradiation with ultraviolet-A (UV-A) light in the presence of 8-methoxypsoralen, and reinfusion of the treated cells into the patient. The mechanism of action has not been fully illuminated. Current knowledge suggests that extracorporeal photo chemotherapy is an amplifier of the immunogenicity of class I-associated peptides that are present on the surface of the collected mononuclear cells. It has been approved by the US FDA (Food and Drug Administration) for the treatment of cutaneous T-cell lymphoma, and encouraging results have been reported in the management of non-malignant disorders of the immune system such as PV, scleroderma, systemic lupus erythematosus, rheumatoid arthritis, autoimmune diabetes mellitus, rejection of cardiac and renal allograft, and chronic graft versus host disease. There are a few case series of patients with PV treated of extracorporeal photo chemotherapy, with most of the patients exhibiting significant clinical improvement and no adverse effects. Research advances have expanded the therapeutic arsenal against pemphigus

vulgaris, which now includes treatments with plasmapheresis, immune specific immune adsorption, extracorporeal photopheresis with exposure of serum to psoralens and UVA, antagonists of tumour necrosis factor alpha (TNF-alpha), Cholinergic antagonists and anti-CD20 monoclonal antibodies (E.g., Ritumaxib). [(22)]

However, no treatment has demonstrated superiority over the others. In fact, there is a lack of well-designed studies on the efficacy of the numerous new PV treatments and a shortage of evidence-based clinical guidelines. This can largely be attributed to the low frequency of the disease and a failure to establish a consensus on terms used to describe and analyse the extent, activity, severity, or healing and remission of PV or on time points for assessing the therapeutic response. Finally, a close collaboration between dentists and dermatologists is required to combat this disease.

Conclusion:

In the last few decades, there have been significant advances in the understanding in the pathogenesis of pemphigus. The knowledge has been transformed into improvement in diagnosis with the use of immunofluorescence, immune precipitation, immune blotting, ELISA. There is also improvement in treatment modalities with decrease in mortality by the use of immune suppressants and improved disease monitoring. But there are also a number of crucial questions remained to be answered. These include the mechanism leading to immune intolerance of self-antigen, role of acetylcholine antibodies, and mechanism of acantholysis by antibodies.

Azathioprine and MMF are often considered first-line therapies for PV with good improvement. Rituximab is beneficial in patients who have poorly controlled disease despite high-dose steroids or steroid-sparing agents (or both) or are contra-indicated for receiving steroids. Intravenous immunoglobulins in short-term studies were effective for recalcitrant cases but its duration of treatment needs further investigation. [(130)] There is no doubt that further research with larger RCTs is required. As more studies incorporate the definitions of disease and therapeutic end-points of the recent consensus statement, it is hoped that valuable and meaningful meta-analyses will provide more definitive answers.

Further research in these areas may provide opportunity for the generation of new treatment strategies and/or prevent the disease occurrence in susceptible individuals. The presently available pathogenesis of PV is rapidly being unravelled, and the search for etiological factors may soon bear fruit. Because of the rarity of the disease, studies are often underpowered and fail to demonstrate a statistically significant difference between the active and control groups. The evidence to date indicates that adding an adjuvant to steroids has a significant steroid-sparing effect, reducing the cumulative exposure to steroids. New, more effective, more specific, and safer treatments are emerging, over and above the recent newer immunosuppressive agents such as mycophenolate and tacrolimus, and include proteinase inhibitor, chimeric molecules for specific recognition and elimination of the autoimmune B-cells, and suggestions for a novel avenue for the development of a non-steroidal treatment for PV using the anti-acantholytic activity of cholinergic agonists.

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MEDICINAL PLANTS WITH NEPHROPROTECTIVE ACTIVITY IN NORTHEAST INDIA

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Abstract:

Northeast India is the paradise of nature is a wonderful reservoir of abundant medicinal plants which are extensively used to treat or cure and prevent various ailments by different tribes or ethnic groups of people. Even though many such medicinal plants are yet to be scientifically proven for their medicinal use, a significant number of medicinal plants of Northeast India have been scientifically evaluated for their ailment resisting or curing potential. In this review, it will be discussed medicinal plants of Northeast India which have scientifically been proven to possess the capability of nephroprotectivity, based on the fact that people with kidney diseases are increasing slowly in Northeast India and around the globe.

Keywords: kidney, nephrotoxicity, renal pathology, acute kidney injury, chronic kidney disease, renal failure.

Abbreviations used:

NSAIDs	Non-steroidal anti-inflammatory drugs
ACE	Angiotensin-converting enzyme
RCD	radiographic contrast dye
CKD	Chronic kidney disease
NCD	Non-communicable disease
ATP	Adenosine triphosphate

Introduction:

Northeast India is a state rich in biodiversity and a landscape full of flora and fauna, including many endangered and rare species. Being part of eastern Himalayan biodiversity of India, Northeast India accounts for 35.48% forest area. In Northeast India, there are many ethno medicinal plants which are extensively used as traditional medicine to treat different diseases for example, jaundice, hypertension, skin disease, respiratory disease, kidney disease etc. by different tribes who are endogenous to Northeast India. Many of these plants are still to be assessed and evaluated scientifically to know about their active compounds and other phytochemical and their role in treating and protecting from a particular disease. Although there are a few medicinal plants of this region which have been scientifically studied about their chemical constituents, their benefits, and different medicinal activities.

Kidney diseases are now a growing concern worldwide currently as it has become one of the principal non-communicable diseases of mortality and a chief factor for cardiovascular disease. Kidney diseases are caused by various factors such as NSAIDs, ACE inhibitors and angiotensin II receptor blockers, Aminoglycoside Antibiotics, radiographic contrast dye (RCD),

heavy metal. Family history comprising CKD patient, gender, ethnicity, race, obesity, socioeconomic status, smoking, nephrotoxins, acute kidney injury, diabetes mellitus, hypertension are major risk factors of chronic kidney disease leading to severe renal impairment. These risk factors are now rising among human populations globally on account of advancement of lifestyle. Hence, patients with kidney diseases are also rising simultaneously. Recent published data also shows that nationwide India paralleling with Northeast India is witnessing a rise in patients with kidney diseases.

The mortality rate caused by kidney diseases is higher in low- and middle-income countries like India, down to less treatment facility and accommodations, high cost of medicines and poor socio-economic status. Several synthetic drugs used to treat these diseases usually have some side effects that adversely affect the health of the patient. Hence, as an alternative to these drugs, it is necessary to discover more natural drugs to treat such disease by determining potential phytochemical and bioactive compounds in medicinal plants.

Northeast India is a promising place for medicinal plants and hence, Northeast India can become prime study area for scientific evaluation and validation of medicinal plants found in this area and can contribute to the discovery of more natural drugs to fight against kidney diseases.

Causes of renal failure and kidney diseases:

Factors for renal failure may fall into two categories–intrinsic and extrinsic. Obesity and non-communicable diseases (NCD) such as diabetes, heart disease, liver, and lung damage could be considered as the extrinsic factors while kidney stones, renal fibrosis, and polycystic condition of kidney. are categorized as intrinsic factors. Family history comprising CKD patient, gender, ethnicity, race, obesity, socioeconomic status, smoking, nephrotoxins, acute kidney injury, diabetes mellitus, hypertension are major risk factors of chronic kidney disease leading to severe renal impairment. Also, family history comprising CKD patient, gender, ethnicity, race, socioeconomic status, smoking, hypertension are major risk factors of chronic kidney disease leading to severe renal impairment. Multiple nephrotoxic drugs such as some drugs used in cancer treatment apart from these factors, NSAIDs, ACE inhibitors and angiotensin II receptor blockers, Aminoglycoside Antibiotics, radiographic contrast dye, environmental pollutants such as heavy metals and some natural nephrotoxicants have the potential to cause nephrotoxicity by involving complex pathophysiological mechanism facilitated through alteration of intraglomerular hemodynamic, impaired tubular secretion and inflammation

Prevalence of kidney diseases, renal injury:

Globally, renal injury caused by chronic kidney disease is the 12^{th} chief cause of death and the 17^{th} cause of disability. Kidney disease is a global public health problem, affecting over 750 million persons worldwide. The prevalence of CKD is higher in older individuals than in younger ones. In terms of gender, the prevalence of CKD is more in women than men. Moreover, CKD is more common in people with diabetes mellitus and hypertension than people with another lifestyle or other NCDs. Also, the prevalence of CKD is advanced in low- and middle-income countries.

There was a rise of 29.3% in global all age prevalence and mortality caused by CKD hiked by 41.5% between the periods of 1990 to 2017. Notably, there was a rise of 38% mortality owing to kidney failure in India between 2001 to 2003 and 2010 to 2013. The incidence of CKD is drastically increasing in Northeast India, a North Eastern state of India, in the recent past

Overview of the mechanism of kidney disease:

The study of mechanism underlying kidney disease is a multidimensional study as kidney diseases are caused by different factors. It is noteworthy that nephrotoxicants mostly target the proximal tubule of nephron. The account of kidney damage and the parameters of its staging are similar in renal injury caused by both nephrotoxicants and renal pathology. Most of nephrotoxicants or compounds induce necrosis, apoptosis, and autophagy in the kidney. The mitochondria in the proximal tubule of nephrons mediate cell death by releasing pro-apoptotic inducers and the produces ATP, which principally regulates whether the cell will die by apoptosis, necrosis, or autophagy. The mechanism of kidney disease caused by nephrotoxicants and renal pathology is amazingly similar in sufficient aspects. Kidney disease caused by both factors induces renal cell death and brings variations in the structure of nephron. Loss of nephrons or any part of nephron can alter physiological activity of the human body significantly.

Medicinal plants of northeast india with nephroprotective activity

Northeast India houses many medicinal plants and herbs which are grown in wild and also reared by natives. These plants have awesome health benefits and successful medicinal potential for ages. Consumption of such medicinal plants in different forms helps in preventing and treatment of diseases. Some of the common medicinal plants with nephroprotective activity are given below in table 1.

Northeast India is a reservoir of many medicinal plants as well as herbs. Many of these plants and their ethno medicinal claim are acknowledged only to the native people and the tribes in Northeast India for a long time. A vast majority of such ethno medicinal plants have not yet been discovered experimentally. The existing active compounds of these medicinal plants of Northeast India may be used for designing new natural drugs with pharmaceutical potential to protect against kidney diseases, thus providing humankind an alternative medicine. Such alternative plant-based drug is reported to have few or no side effects under a specific dose hence, these have emerged as an alternative to allopathic medication. In this review, a few of the ethno medicinal plants which have been experimentally reported to possess nephroprotective activity are discussed. This study can put light to further scientific evaluation to discover new natural plant-based drug and thus pave a new path in pharmaceutical research area and may be a boon for the humankind by protecting a section of human population against detrimental kidney diseases.

Table 1: Some reported medicinal plants of Northeast India with nephroprotective activity, parts of the plants used for experimental study, their local name, and solvent used for extract preparation

Sl no.	Medicinal plants	Part used	Local name in Northeast India	solvent	References
1	*Momordica dioica*	rhizome	Bhat kerela	Ethyl acetate	Talukdar *et al.* (2018)
2	*Garcinia pedunculata*	fruit	Bor thekera	ethanol	Ravi *et al.* (2017)
3	*Murraya koenigii*	leaf	Narasingha	Aqueous	Ghosh *et.al.* (2015), Ghosh *et al.* (2012)
4	*Scoparia dulcis L.*	aerial parts	Bon-dhonia/ Bon chini/ Modhumehari.	Ethanol	Jose *et al.* (2015), Sarmah *et al.* (2021)
5	*Annona squamosa L*	leaf	Attaphol, Atlas, Ata kothal, Sita phol	ethanol	Neelima *et al.* (2020)
6	*Azadirachta indica*	leaf	Neem	Methanol	Moneim *et al.* (2020)
7	*Citrullus colocynthis*	fruit	Kuwa baturi	Ethyl alcohol	Atef *et al.* (2011)
8	*Momordica charantia L.*	fruit	Tita kerela	Ethanol	Jain *et al.* (2010)
9	*Solanum xanthocarpum*	Fruit	Tita bekhuri	Ethanol	Hussain *et al.* (2012)
10	*Phyllanthus emblica*	Leaf	Amlokhi	Ethanol	Purena *et al.* (2018)
11	*Vigna mungo L*	Seed	Matimah	Aqueous, methanol	Ifthekar *et al.* (2012)
12	*Ficus hispida(L.f)*	Fruits	Jagya dumabru	methanol	Swathi *et al.* (2011)
13	*Andrographis peniculata. (Burm.f)*	Whole plant	Kalmegh.	Aqueous	Singh *et al.* (2009)
14	*Syzygium cumini (L.)*	seed	Kola jamu	Aqueous	Behera *et al.* (2014)
15	*Abrus precatorius*	Whole plant	Latumoni	Chloroform	Kumari *et al.* (2021)

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UNMASKING THE SILENT THREAT: EXPLORING THE RAVAGES OF CHRONIC TRAUMATIC ENCEPHALOPATHY (CTE)

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Abstract:

Chronic Traumatic Encephalopathy (CTE) is a progressive neurodegenerative disorder associated with repeated head trauma, particularly prevalent in individuals engaged in contact sports or professions with high risks of head injuries. This chapter provides an in-depth examination of CTE, encompassing its etiology, pathological characteristics, and clinical manifestations. The stages of CTE and their corresponding symptoms are elucidated, outlining the progressive nature of the disease. Diagnostic challenges and the current utilization of imaging biomarkers for CTE detection are explored. Furthermore, the chapter investigates the link between early participation in contact sports and the earlier onset of CTE. Enhancing our comprehension of CTE is vital for refining preventive strategies, raising awareness, and developing effective treatment modalities to mitigate the profound impact of this debilitating condition.

Keywords: Axonal Injury, Phosphorylation, Encephalopathy.

Introduction:

In the field of neurology, there exists a hidden threat that gradually erodes the core of a person's brain known as Chronic Traumatic Encephalopathy (CTE). Chronic traumatic encephalopathy (CTE) is a condition characterized by distinct neuropathological features observed in the brains of individuals who have a history of repetitive traumatic brain injury (TBI) and are examined postmortem [1]. CTE is characterized by the abnormal buildup of hyperphosphorylated tau protein (p-tau) around blood vessels, initially occurring in the crevices of the brain's outer layer and gradually spreading throughout. However, the neuropathology of CTE extends beyond just p-tau aggregation. It is a multifaceted neurodegenerative disorder that involves various brain pathologies, including dysfunction in neurovascular processes, damage to axons, inflammation in the nervous system, and accumulation of abnormal proteins [2]. Researchers have discovered that in cases of multifocal traumatic axonal injury, the most prevalent occurrence is the presence of clusters of p-Tau (phosphorylated tau protein) in the form of neurofibrillary tangles, pre-tangles, and neurites, primarily located around blood vessels [3]. This condition has been observed in athletes participating in various sports such as American football, soccer, rugby, ice hockey, lacrosse, mixed martial arts, wrestling, and boxing, both at amateur and professional levels. It has also been identified in military veterans, victims of domestic violence, individuals with a history of multiple falls, and those who engage in head-banging behaviour [4]. At present, the diagnosis of CTE can only be confirmed through

postmortem examination of brain tissue, which poses a limitation for conducting extensive prospective clinical studies to determine the occurrence and prevalence of CTE in the general population. This obstacle also hampers efforts to comprehend the progression of the disease in living individuals and prevents personalized medical intervention for individuals with a higher risk of developing CTE [2].

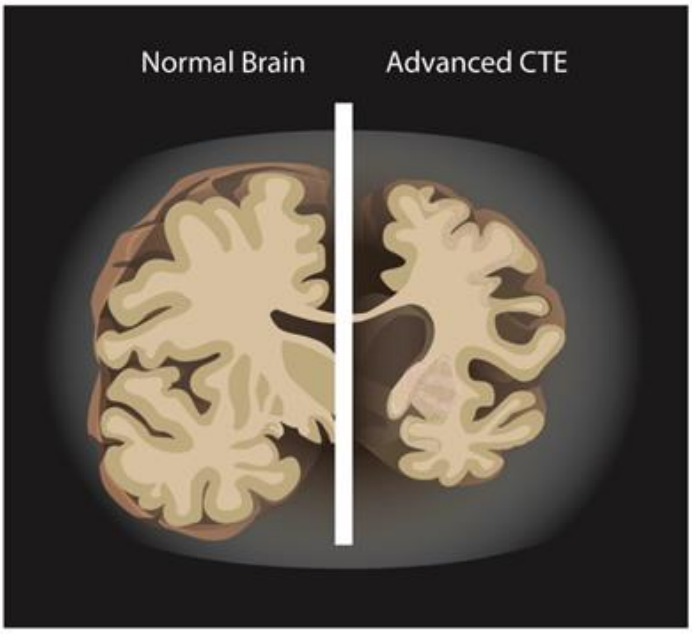

Fig. 1: Chronic Traumatic Encephalopathy

Neuropathology:

Multiple brain pathologies are involved in repetitive brain injury leading to chronic traumatic encephalopathy, which are:

A. Neurovascular Dysfunction

The neurovascular unit (NVU) is a well-structured assembly of cells that includes endothelial cells, pericytes, astrocytes, and neurons. Its primary role is to regulate blood flow in the brain based on neural activity. Additionally, the NVU plays a crucial role in maintaining the integrity of the blood-brain barrier (BBB), which is essential for providing neurons with sufficient oxygen and energy substrates to support efficient synaptic transmission [5]. Astrocytes play a vital role in facilitating the brain's waste clearance mechanism called the glymphatic system. Within this system, there exists a crucial component known as aquaporin-4 (Aqp4), which is a water channel predominantly found in astrocytes [6]. The glymphatic system operates by enabling the entry of cerebrospinal fluid into the brain through arteries and subsequently clearing waste through the veins. Aqp4, found primarily on astrocytic end feet near blood vessels, plays a role in regulating the flow of interstitial fluid. This fluid carries waste products such as tau protein and β-amyloid peptides out of the brain, facilitating proper waste clearance.

However, following head trauma, astrocytes undergo changes and enter a reactive state, resulting in increased production of inflammatory substances. This reactive state leads to the displacement of Aqp4 from the astrocytic end feet, disrupting the glymphatic clearance process. Consequently, harmful proteins begin to accumulate in the brain. Experimental head trauma can

also impede glymphatic flow, resulting in the aggregation and buildup of p-tau protein [2]. Furthermore, when astrocytes become reactive, they can disrupt interactions with endothelial cells and neurons within the neurovascular unit (NVU). This disturbance leads to impaired NVU function, resulting in reduced blood flow to the brain. Consequently, local neural circuits are deprived of the necessary energy for normal signalling, leading to neuronal decline and impaired brain function. Additionally, reactive astrocytes have been found to compromise the integrity of the blood-brain barrier (BBB) by increasing the expression of vascular endothelial growth factor-A, a protein that attracts cells and influences the tight junctions between endothelial cells [2].

B. Axonal Injury

Axonal injury in repetitive head trauma can have profound effects on the nervous system. Neuronal axons, responsible for transmitting nerve signals, are highly vulnerable to mechanical deformation and stretching during traumatic events. This physical strain can disrupt ion balance within neurons, triggering cascades of events that lead to further damage. Calcium and potassium fluxes are altered, resulting in spreading depolarization, seizures, and the release of excitatory neurotransmitters like glutamate. These excitotoxic processes, combined with oxidative stress, contribute to neuronal injury and impaired brain function. CTE is associated with axonal injury, although the precise mechanisms remain unclear. Traumatic axonal injury (TAI) is a consistent feature observed in CTE. Neuropathological examinations reveal axonal retraction balls and swellings in various brain regions affected by repeated head trauma. The severity of brain injury correlates with the presence and extent of TAI. Axonopathy plays a crucial role in CTE, and its severity aligns with the degree of neurodegeneration. The accumulation of abnormal tau protein is associated with reduced axonal integrity. Axonal injury in CTE may trigger abnormal tau deposition, but the process likely involves a bidirectional relationship, where tau accumulation contributes to further neurodegeneration. Understanding these mechanisms is essential for developing interventions to mitigate the long-term consequences of repetitive head injuries [2].

C. Neuroinflammation

Neuroinflammation is an immune response in the brain that persists long after retirement in contact sport athletes. Increased uptake of translocator protein ligands, detected through imaging, suggests chronic neuroinflammation in retired National Football League players. Postmortem studies have shown elevated CD68-reactive microglia staining in the frontal cortex of individuals with a history of repetitive head injury. Furthermore, the severity of neuroinflammation in contact sport athletes with confirmed CTE correlates with disease severity. Microglia, the primary inflammatory regulators in the brain, possess various receptors that respond to brain injury. After experimental head injury in mice, microglia adopt a specific TREM2+ phenotype associated with immune response and tissue repair. Disruptions in microglia-neuron communication and damage to the neurovascular unit lead to neuroinflammation. Leakage of proinflammatory plasma proteins into the brain parenchyma, caused by loss of blood-brain barrier integrity, stimulates microglia and activates astrocytes. These proteins have been found in the brains of young contact sport athletes shortly after injury [12].

D. Tau phosphorylation

The hallmark diagnostic feature of CTE is the abnormal accumulation of hyperphosphorylated tau protein around small blood vessels in the depths of cortical sulci. Tau protein is normally involved in maintaining the stability of neuronal axons and dendrites. However, in certain pathological conditions, tau protein undergoes abnormal hyperphosphorylation, leading to its dissociation from microtubules and subsequent aggregation into toxic forms, such as neurofibrillary tangles (NFTs). This abnormal tau aggregation disrupts cellular processes, including protein trafficking, neuronal transport, synaptic function, and increases neuronal hyperexcitability.

Tau phosphorylation can occur at multiple sites by various kinases, resulting in different forms of tau with varying aggregation propensity and neurotoxicity. Specific phosphorylated tau residues, such as Ser202 and Ser202/Thr205, serve as markers of early pathology and mature NFTs, respectively. Additionally, a recently discovered form called cis-p-tau, detected in human brains and animal models after traumatic brain injury (TBI), has been found in cases of CTE and Alzheimer's disease. Cis-p-tau appears early after TBI and can persist for several months. Importantly, experimental studies have shown that targeting cis-p-tau with therapeutic monoclonal antibodies can alleviate neurocognitive deficits, neurodegeneration, and tauopathy induced by neurotrauma [2].

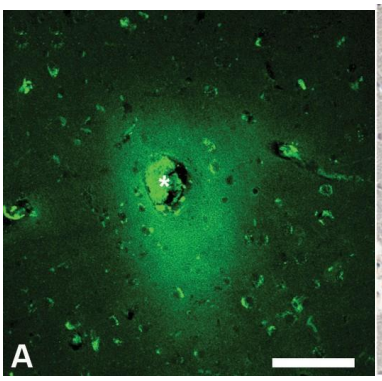

Fig. 2: Neurovascular Dysfunction

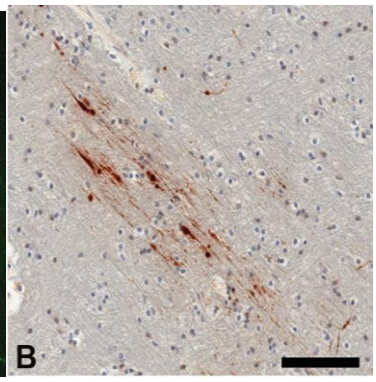

Fig. 3: Axonal Injury

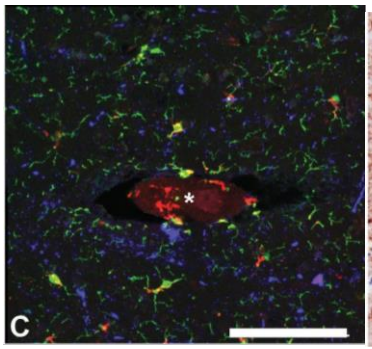

Fig. 4: Neuroinflammation

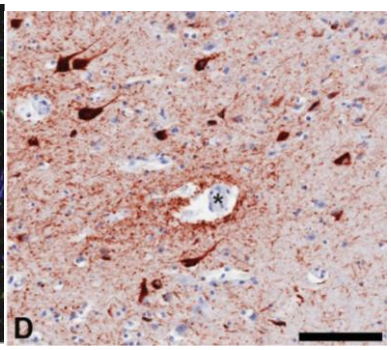

Fig. 5: Tau Phosphorylation

Epidemiology

According to the 2015 NINDS-NIBIB consensus pathologic criteria for CTE, a review of 1,721 cases from the Mayo Clinic Brain Bank showed that contact sport athletes had CTE at a rate of 32%. No cases of CTE were found in 162 control brains without a history of traumatic

brain injury (TBI) or contact sports, and there were no instances of CTE among 33 individuals with a single TBI. In the largest case series on CTE to date, Mez and colleagues reported that out of 202 deceased individuals with a significant history of repetitive head trauma from contact sports or military service, 177 former professional football players (87% of the cases) and specifically 110 out of 111 former National Football League (NFL) players (99% of the cases) were diagnosed with CTE. The study acknowledged that there was a bias in participant selection as individuals with symptoms indicative of possible CTE were more likely to participate in the brain donation program. Hence, the true frequency of CTE pathology remains unknown [8].

Clinical presentation

Chronic traumatic encephalopathy (CTE) is a neurodegenerative condition that has gained significant attention due to its association with repeated head injuries, particularly in contact sports. This chapter aims to provide an in-depth understanding of the clinical presentation of CTE. By exploring its cognitive, behavioural, and physical manifestations, readers will gain valuable insights into this complex condition [9].

A. Cognitive symptoms:

- Memory Loss: Difficulty recalling recent occurrences or retaining new knowledge.
- Impaired Attention and Concentration: Trouble focusing or staying attentive for extended periods.
- Executive Dysfunction: Challenges with planning, decision-making, and organizing tasks.
- Language Impairment: Difficulty with language-related abilities, such as finding the right words or understanding complex sentences.
- Visuospatial Difficulties: Problems with spatial awareness and visual perception.

B. Behavioural and mood changes:

- Depression and Anxiety: Persistent feelings of sadness, hopelessness, and worry.
- Irritability: Easily becoming annoyed or agitated.
- Aggression: Displaying hostile or violent behavior.
- Impulsivity: Acting without thinking through the consequences.
- Personality Changes: Alterations in one's overall demeanor, temperament, or behavior.
- Insomnia: Difficulty falling asleep or staying asleep.
- Feeling of Hopelessness: Overwhelming sense of despair or lack of optimism.
- Sudden Highs and Lows: Experiencing extreme mood swings.

C. Physical symptoms:

- Headaches: Recurrent or persistent head pain.
- Dysarthria: Difficulty controlling the muscles involved in speech, leading to slurred or unintelligible speech.
- Spasticity: Increased muscle tone, resulting in stiffness and spasms.
- Gait Impairment: Abnormalities in walking, such as unsteadiness or difficulty with coordination.

McKee's four stages of cte

Stage I CTE:

In this stage, the brain appears normal grossly, but abnormal tau protein (p-tau) is found in specific areas, such as the lateral and frontal cortices, and close to small blood vessels within brain crevices. There may be a limited presence of neurofibrillary tangles (NFTs) and neurites in the locus coeruleus, a brainstem region.

Stage II CTE:

Macroscopic abnormalities become localized, with observable changes in brain sections and neuroimaging. These include enlarged lateral ventricles, cavum septum pellucidum (a fluid-filled structure in the brain) with or without fenestration, and paleness in the locus coeruleus and substantia nigra. There is an increased number of p-tau foci within brain crevices, with a spreading pattern emerging.

Stage III CTE:

Gross pathological sections exhibit more widespread macroscopic abnormalities. There is overall brain weight loss, mild atrophy (shrinkage) of the frontal and temporal lobes, and ventricular dilation. Approximately 50% of individuals diagnosed with chronic traumatic encephalopathy (CTE) exhibit septal abnormalities, which may include the presence of a cavum septum pellucidum. P-tau pathology spreads further, affecting the frontal, temporal, parietal, and insular cortices.

Stage IV CTE:

Brain weight reduction becomes drastic, with reported brain weights as low as 1,000 g compared to the normal range of 1,300-1,400 g. Profound atrophy is observed in the frontal and medial temporal lobes, as well as the anterior thalami. White matter tracts also experience atrophy [13].

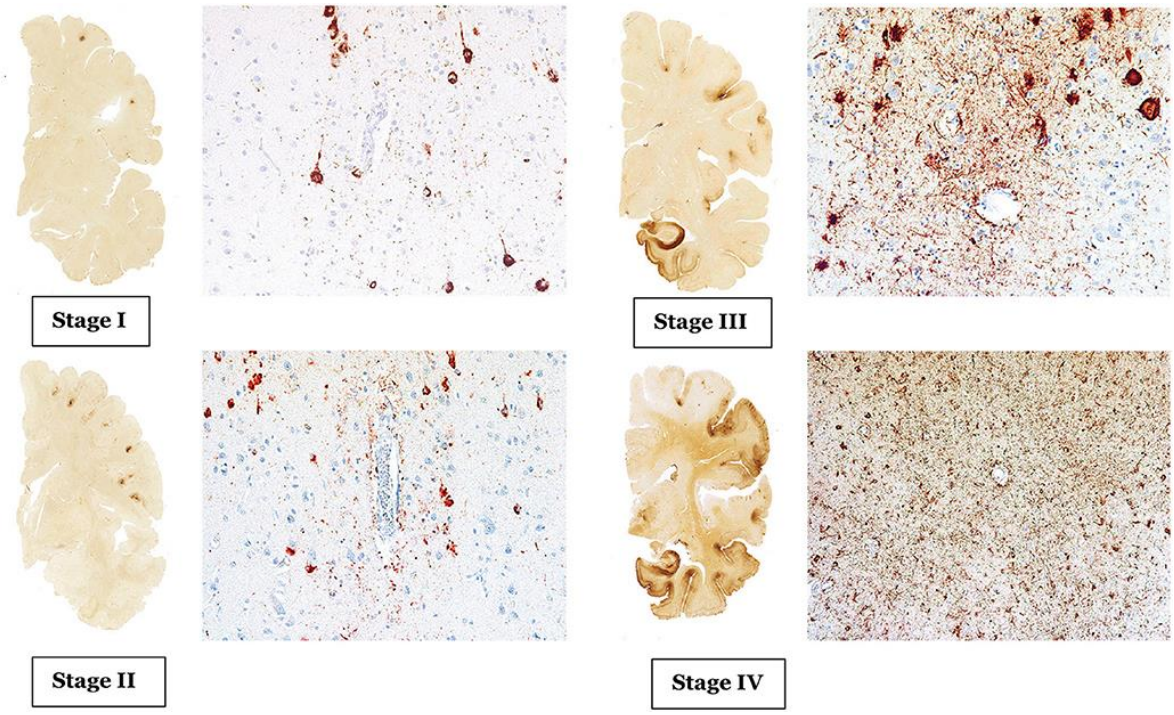

Fig. 6: McKee's Stages of CTE

Diagnosis

Clinical diagnostic criteria

Five criteria to make clinical diagnosis:

1. A documented history of multiple head impacts is required.
2. Symptoms should not be attributable to any other underlying condition.
3. Symptoms must be present for a minimum of 12 months.
4. At least one core clinical feature (mood, cognitive, or behavioural impairment) should be evident.
5. Supportive features such as a decline over 12+ months, headaches, or impulsivity should be present.

Imaging

1. Diffusion Tensor Imaging - Assessment of the integrity of white matter in the brain.
2. Functional MRI - Identification of variations in hemoglobin oxygenation along with correlation to brain activity during specific tasks.
3. Positron emission tomography (PET) scans - Utilization of imaging biomarkers (FDDNP, T807, AV1451, and flortaucipir) in detecting CTE. Uptake of these biomarkers in specific brain regions depends on the particular biomarker being used.

Fluid biomarkers

1. t-tau – It Indicates Neuroinflammation, ER abnormalities and Oxidative stress.
2. sTREM2 – Marker of Microglial Activation
3. Chemokine – It is an inflammatory marker.
4. Neurofilament Light Chain – It is increased in axonal injury.
5. Glial Fibrillary Acidic Protein – Glial derived indicator [10]

Risk factors

CTE is strongly linked to a history of repetitive head trauma, such as multiple concussions or mild traumatic brain injuries (TBIs). This includes both recognized concussions and sub concussive blows that may not have been diagnosed or reported. [14]

The primary determinant of increased susceptibility to CTE is the cumulative and repetitive occurrence of head impacts.

- Sub concussive blows are impacts to the head that are forceful enough to affect the integrity of neurons but do not cause recognizable concussion symptoms.
- The number of hits sustained per football season.
- Engaging in contact sports at a young age is associated with an earlier onset of chronic traumatic encephalopathy (CTE).

Treatments

The current approach to treating Chronic Traumatic Encephalopathy (CTE) primarily revolves around providing supportive care. However, advancements in treatment development have emerged as a result of recent insights into neurobiological mechanisms gained from studies conducted on rodent models.

Supportive therapy

- Cognitive rehabilitation therapy - This therapy helps improve cognitive skills like memory, attention, problem-solving, and decision-making that may be affected by CTE.
- Motor Therapy - Motor therapy focuses on addressing motor problems such as coordination, balance, and fine motor skills. It helps individuals with CTE improve their physical movements.
- Mood and behaviour therapy - This therapy assists in managing mood and behavior issues like depression, anxiety, irritability, and aggression often associated with CTE.
- Mindfulness - Mindfulness practices like meditation and breathing exercises are used to reduce stress, anxiety, and promote overall well-being in individuals with CTE.
- Mediterranean diet - While not a cure, following a Mediterranean-style diet rich in fruits, vegetables, whole grains, lean proteins, and healthy fats may have benefits for brain health in CTE.
- Aerobic exercise - Regular aerobic exercise, like brisk walking or cycling, can improve cognition, mood, and overall brain health in individuals with CTE.
- Vestibular rehabilitative therapy - This therapy helps address problems with balance, dizziness, and vertigo by utilizing exercises and techniques to improve spatial orientation.
- Occupational-Ocular Therapy: This therapy focuses on improving visual and eye-related impairments in individuals with CTE, such as eye-hand coordination and visual processing.

Symptomatic medication

- Memory impairment medications—galantamine, donezepil, and rivastigmine
- Stimulants—methylphenidate
- Dopamine agonists—carbidopa/levodopa, pramipexole, amantadine, memantine
- Antidepressive/anxiety medications—sertraline and escitalopram

Potential treatment

- Targets tau acetylation - Tau acetylation refers to a chemical modification of the tau protein in the brain, which plays a role in the development of abnormal tau aggregates seen in CTE. Therapies targeting tau acetylation aim to inhibit or reverse this modification, potentially preventing or reducing tau-related pathology in CTE.
- Targets tau phosphorylation – Tau phosphorylation refers to the addition of phosphate groups to the tau protein, which can lead to the formation of tau tangles in CTE. Kinase inhibitors are medications or compounds that specifically target and inhibit the activity of enzymes called kinases, which are responsible for tau phosphorylation. By blocking or reducing the phosphorylation of tau, these inhibitors may help mitigate the progression of tau pathology in CTE.
- Immunotherapy – Anti p-tau Antibody and 6C5: Immunotherapy involves using antibodies or other immune-based approaches to target and remove specific proteins involved in disease pathology. In CTE, antibodies targeting phosphorylated tau (p-tau) or a specific antibody called 6C5 can bind to and help clear abnormal tau aggregates. This

approach aims to reduce tau pathology and potentially slow down the progression of CTE.

- Inflammation Targeting – salubrinal, Calpain 22 inhibitor and 2-AG plays a significant role in the development and progression of CTE. Therapies targeting inflammation aim to reduce the inflammatory response in the brain. Salubrinal, calpain 22 inhibitors, and 2-AG are examples of compounds that can modulate or reduce inflammation in CTE. By targeting inflammation, these compounds may help protect brain tissue and alleviate some of the detrimental effects associated with CTE [10,11].

Conclusion:

Chronic Traumatic Encephalopathy (CTE) represents a significant concern within the fields of neurology and sports medicine, prompting extensive research to uncover the devastating consequences of repetitive head trauma. The identification and characterization of the stages of CTE, along with their associated symptoms, provide a valuable framework for understanding the progressive nature of the disorder. The presence of neuroinflammation, endoplasmic reticulum abnormalities, and oxidative stress further contribute to the intricate pathophysiology of CTE. Overcoming diagnostic challenges in detecting CTE during an individual's lifetime necessitates advances in imaging techniques and the exploration of potential biomarkers. Additionally, the compelling evidence linking early participation in contact sports to an accelerated onset of CTE underscores the significance of implementing safety measures and promoting awareness. Continuous collaborative efforts among healthcare professionals, researchers, and sports organizations are essential for comprehensively addressing the impact of CTE and formulating effective strategies for prevention, diagnosis, and treatment. By prioritizing the well-being of athletes and individuals at risk, we can strive to minimize the long-term consequences of CTE and ensure a safer future for those engaged in sports and activities prone to head injuries.

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THE FUTURE OUTLOOK FOR MICRONEEDLE DRUG DELIVERY IN DIABETES

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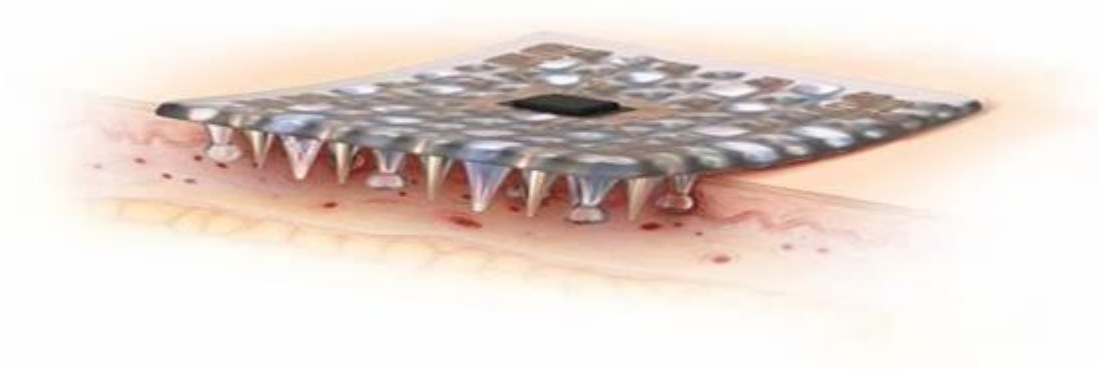
Abstract:

Diabetes is a severe and potentially fatal disease that affects people all over the globe. As a result, there is a need for a more effective and safe way to deliver insulin than the standard delivery technique. Transdermal drug delivery systems are an appealing alternative because they provide strong patient compliance as well as the opportunity for controlled release throughout time while avoiding potential degradation owing to the digestive system or first-pass liver effects. Micro needles are a unique way to increase transdermal medication distribution because they break down the skin and generate micrometre-scale holes. Though clinically quite small, micrometre-sized channels are far larger than molecules and hence should significantly boost the skin's accessibility to large medicinal molecules. They are simple to give and have high patient compliance. Micro needles have proven to be particularly effective in patients with needle fear and adverse effects such as discomfort at the site of management, irritation, tissue necrosis, and others. This paper discusses numerous types of micro needles, their production, materials employed, evaluation factors, and their utility in delivering insulin and other applications.

Keywords: Microneedle, Diabetes, Novel approach, Efficacy

Introduction:

Microneedles



Transdermal drug delivery offers a clinical superiority over traditional, invasive injections. As compared to oral delivery, protein drug transportation across the skin avoids the hepatic first-pass extraction and is delivered to the systematic circulation at a pharmacologically relevant rate. However, the clinical application of transdermal delivery has been limited to lipophilic drugs with a molecular weight less than 500 Da until the emergence of polymeric microneedles (MNs), which provides a broad and versatile platform to overcome the challenges

of the skin barrier for macromolecular drugs. To date, MNs have been applied in the delivery of distinct cargoes, from native protein therapeutics to nano- or micro particle (MP)-based formulations. Of these models, biocompatible polymer-based MN devices have been leveraged to address several issues in transdermal protein delivery [1].

Brief information on microneedles drug delivery system

Hypodermic needles and topical creams are most commonly used when it comes to delivery of the drug through the skin. Patients due to pain associated with them less accept needles and topical creams show less bioavailability. The stratum corneum layer behaves like a major barrier, as it allows only certain molecules like lipophilic and low molecular weight drugs to pass through it [2].The microneedles (MNs) have been studied by various researchers for delivering drug through the transdermal route and for overcoming the limitations of the conventional approaches. Microneedle device consists of needles of micron size, which are arranged on a small patch. Considering the problems of the hypodermic needle and the transdermal patch, microneedle drug delivery system was developed and is thought to be the hybrid of both.

The major problem associated with transdermal technology is that many of the drugs are not able to cross the skin at the required rate necessary for the therapeutic action. Researchers have developed a refined technology using microneedles, which allow hydrophilic high molecular weight compounds to enter into the stratum corneum. In this technique, arrays of microscopic needles are designed to painlessly transverse the stratum corneum and penetrate the dermis layer at a predetermined depth ranging from 70 to 200 µm, thereby avoiding the stimulation of the nerve endings, improving treatment compliance and patient acceptability. Microneedle technique has been successfully used to deliver a variety of compounds including macromolecules and hydrophilic drugs into the skin. As microneedle system bypasses the stratum corneum barrier of the skin, permeability enhancement of two to four orders of magnitude has been observed for small molecules like calcine and for the relatively larger compounds like proteins and nanoparticles [3].

Microneedles (micron-sized needling system) are intended to pierce the epidermal layer, creating micro-channels in the skin without causing pain, bleeding, or infection. These channels allow therapeutic agents to diffuse into the dermal layer, which is well perfused with blood vessels [4]. The length of microneedles can vary to ensure epidermal penetration, while avoiding stimulation of the nerve fibers or puncturing blood capillaries. Microneedle delivery does not face limitations based on the molecular size, since the channels are markedly larger than the typical therapeutic agents. The first report related to the development of micro needles for topical delivery was published in late 1990s, where the use of micro needles was emphasized in puncturing the skin for the enhanced permeability of the drug “calcein” (molecular weight 623 Da) across the human skin by four folds in vitro. Since then, a lot interest has been developed in this field in terms of both micro needle fabrication and drug delivery.

Advantages of microneedles

1. The major advantage of microneedles over traditional needles is, when it is inserted into the skin it does bypass the stratum corneum, which is the outer 10- 15 μm of the skin. Conventional needles which do pass this layer of skin may effectively transmit the drug but may lead to infection and pain. As for microneedles they can be fabricated to be long enough to penetrate the stratum corneum, but short enough not to puncture nerve endings. Thus reduces the chances of pain, infection, or injury.
2. By fabricating these needles on a silicon substrate because of their small size, thousands of needles can be fabricated on single water. This leads to high accuracy, good reproducibility, and a moderate fabrication cost.
3. Hollow like hypodermic needle; solid— increase permeability by poking holes in skin, rub drug over area, or coat needles with drug.
4. Arrays of hollow needles could be used to continuously carry drugs into the body using simple diffusion or a pump system.
5. Hollow microneedles could be used to remove fluid from the body for analysis – such as blood glucose measurements – and to then supply microliter volumes of insulin or other drug as required.
6. Immunization programs in developing countries, or mass vaccination or administration of antidotes in bioterrorism incidents, could be applied with minimal medical training.
7. Very small microneedles could provide highly targeted drug administration to individual cells.
8. These are capable of very accurate dosing, complex release patterns, local delivery and biological drug stability enhancement by storing in a micro volume that can be precisely controlled [5].

Disadvantages of microneedles

Microneedle delivery does not face limitations based on the molecular size, since the channels are markedly larger than the typical therapeutic agents. It possessed some limitation such as, (1) local irritation, (2) erythema, (3) itching, (4) local oedema may be produced by the drug or other excipients at the site of application especially in the patch formulation. (5) Limited permeability across through the skin may limit the delivery of number of drugs. Various attempts have been made to overcome these limitations and make its conventional route [6].

Mechanism of drug delivery

In the microneedle drug delivery system, the skin is temporarily disrupted. A microneedle device is made by arranging hundreds of microneedles in arrays on a tiny patch (the same as that of a normal transdermal patch available in the market) in order to deliver sufficient amount of drug to give a required therapeutic response. It pierces the stratum corneum thus bypassing the barrier layer. The drug is directly placed in the epidermis or upper dermis layer which then goes into the systemic circulation and shows a therapeutic response on reaching the site of action [7].

Controlled release kinetics

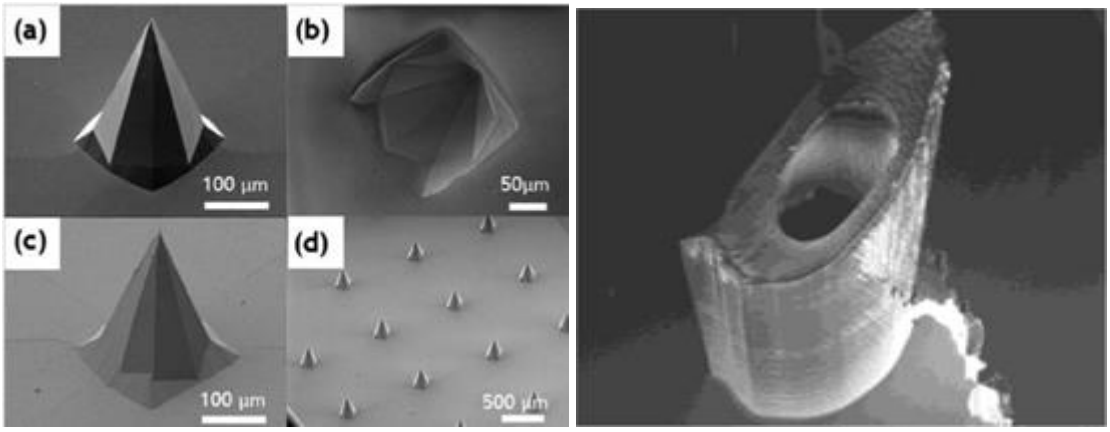
Drug release in polymeric MN systems consists of the transportation of drug molecules from the inner polymeric matrix to its outer surface and their further release into the surrounding tissue. Controlling drug-release kinetics is an important approach to controlled drug delivery. Release kinetics can be manipulated by modifying the polymer or exploiting the intrinsic properties of the drug molecules.

Sustained drug release

The sustained delivery has been pharmaceutically attractive in terms of maintaining the constant range of drug concentration in the body. The sustained delivery with drug loaded microneedles has been attempted by a few different approaches such as lowering the diffusion of drug with additional excipients or less soluble matrix (Park *et al.,* 2006), encapsulating less soluble micro particles in a highly soluble microneedle matrix (Ito *et al.,* 2007a, Donnelly *et al.,* 2010, Kim *et al.,* 2012a), adding the external drug reservoir after the insertion of microneedles (Kolli and Banga, 2008), and utilizing the backing layer of a microneedle patch as an additional drug depot (Lee *et al.,* 2008, Garland *et al.,* 2012a) [8].

Materials used in formulation

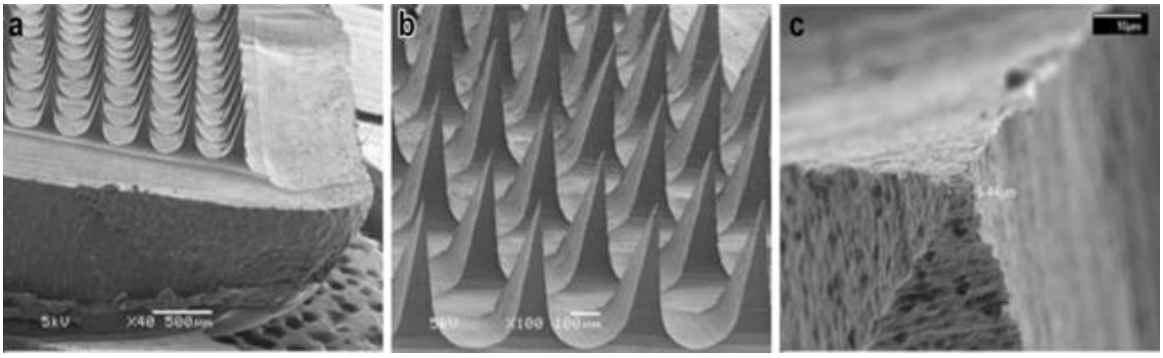
(1) Silicon: Silicon was the material selected for the first MNs used for drug delivery because the technology needed to manufacture micron or submicron structures only became available with the advent of industrial high-precision microelectronics tools during the 1990s. Silicon has proved very useful in manufacture of microstructures and microelectromechanical systems (MEMS) for a number of reasons. Its main advantage is that there is much flexibility in the processes that can be used to shape it, meaning that microstructures in a variety of desirable shapes and sizes can be readily produced [9].



Microneedles fabricated using silicon as material

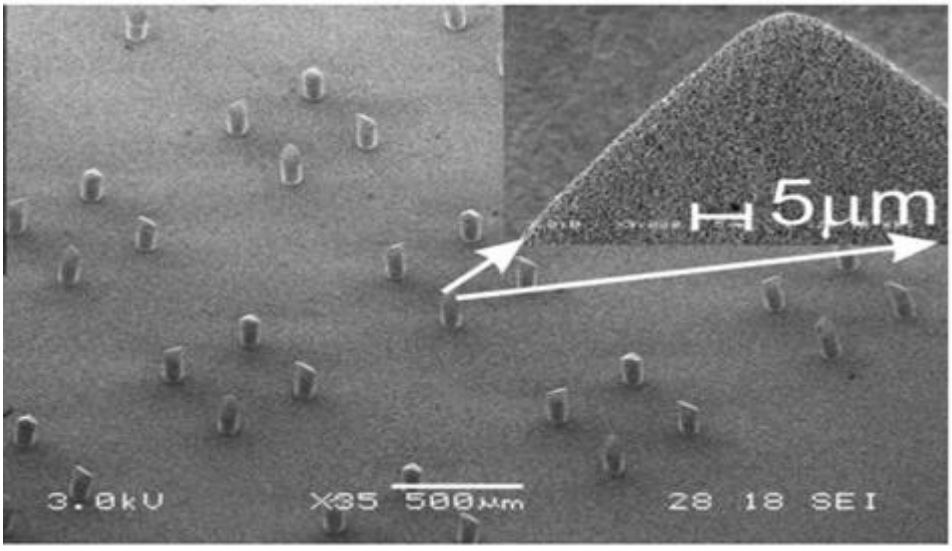
(2) Metals: Although silicon is attractive, as a common microelectronics substrate with extensive processing experience for more than 30 years, it is relatively expensive and requires clean room processing (Banga, 2009) [10]. In contrast, metal and glass MNs have been found to be equally effective in skin penetration and can be produced at relatively much lower cost than silicon MNs. Various metals, such as stainlesssteel, titanium, palladium, palladium-cobalt alloys, and nickel has been used as structural

materials for MN fabrication (Chandrasekaran & Frazier, 2003; Chandrasekaran *et al.*, 2003; Verbaan *et al.*, 2008) [11].



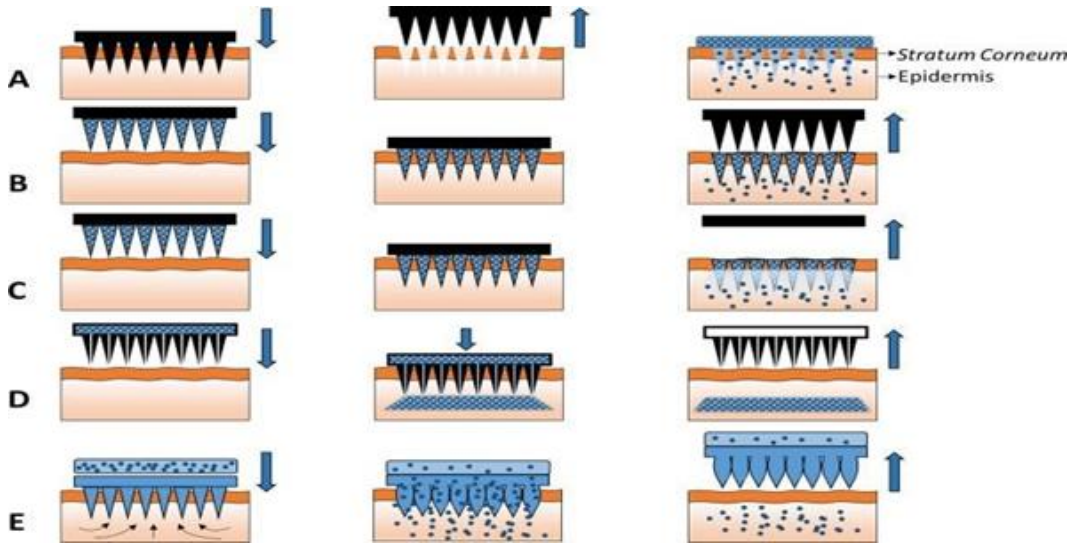
Different microneedles fabricated with metals

(3) Ceramic: Alumina (Al2O3) is mainly used because of its chemical resistance. It forms a stable oxide because of the highly energetic ionic and covalent bonds between Al and O atoms. Other types of ceramics used are calcium sulfate dihydrate [Gypsum (CaSO4 0.2H2O)] and calcium phosphate dihydrate [Brushite (CaHPO4.2H2O)] [12]. Due to their superior chemical properties and compression resistance, ceramic materials such as alumina have been used to fabricate a MN. However, alumina possesses a lower tensile strength compared to other materials [13]. Ceramic microneedles have been also fabricated using techniques such as micromoulding of a ceramic slurry and subsequently sintering of the replicated green body. Ceramic micromoulding techniques offer the advantage to realize device production at a low-cost process due to the potential of up-scaling the technology [14].



Microneedles fabricated with Ceramic as material

(4) Silica glass: Varying geometries can be produced on small scale using glass. Silica glass is physiologically inert but brittle in nature. Borosilicate glass which is made up of silica and boron trioxide is more elastic. They are mostly fabricated manually, thus are less time efficient. Glass MNs are not used now commercially, but only for experimental purposes.



Schematic representation of different types of MNs. (A) Solid MN, (B) Coated MN, (C) DissolvingMN, (D) Hollow MN and (E) Hydrogel forming MN

(5) Polymer: The majority of previously reported work on microneedles fabrication has used silicon as the material of construction. However, due to its brittle nature, metal or hard polymer materials have recently gained considerable interest. Silicon is thus used to produce a master from which an inverted mold is extracted and often sacrificed to reproduce the original master into metal or polymer [15].

Types of microneedles:

The typical design parameters to be considered in the selection of polymeric matrix include biocompatibility, biodegradability, solubility and mechanical properties. Based on the available materials and formulations, the construction of an MN delivery system can be achieved via several main strategies. Gerstel and Place first used the microneedle as a drug delivery system in 1971, which laid a foundation for the application of microneedle systems in transdermal drug delivery systems. Morphologically, microneedles are divided into four types, including solid microneedles, coated microneedles, dissolving microneedles and hollow microneedles. Additionally, microneedles can be comprised of various materials, such as metal materials, inorganic materials and polymer materials. Microneedles of different types and different materials play different roles in different research fields. In recent years, the microneedle delivery system has been widely used to deliver drugs, genes, proteins, RNA and vaccines [16]. Hollow MN are like regular hypodermic needles but shorter in length. A liquid formulation of the drug is infused through bores in the MN. Solid MN are used to create holes in the skin. Subsequently a patch is then applied. Coated MN are MN coated with the drug while polymer MN are made from polymers that can be dissolving, no dissolving or hydrogel-forming [17].

1) Solid microneedles: Solid microneedles are an array containing microscale tapered sharp tips composed of a single material without any drugs or excipients, they are inserted into the skin, creating micron-sized pores on the skin surface. When the drug is placed on the treated area, the

drug passes through the stratum corneum, the largest barrier of the skin, through these pores; it is easily transferred to the capillaries in the superficial dermis, increasing the bioavailability of the drug.

(2) Dissolving microneedles: Dissolving microneedles are composed of biodegradable and biocompatible materials that tend to degrade and dissolve in body fluid leading to the release of loaded cargo. Usually, they are fabricated using the micro-moulding technique, Drug release is mainly controlled through the dissolution rate of materials. These kinds of microneedles have the drawback of dose limitation as compared to solid, hollow, and hydrogel-forming microneedles. Zhao *et al.* reported hyaluronic acid-based fast-dissolving microneedles loaded with 5-Aminolevulinic acid (a precursor of proporphyrin IX) for photodynamic therapy using micro-moulding. They found a fast release in-vitro release (~100 % in 60 min) of 5-ALA using Franz diffusion cell [18].

(3) Coated microneedles: The microneedles are surrounded with the drug solution or drug dispersion layer. Subsequent dissolution of drug from the layer takes place and the drug is delivered quickly. The amount of drug that can be loaded depends on the thickness of the coating layer and the size of the needle which is usually very less. Baek et al loaded lidocaine on poly L-lactide (PLLA) micro needle arrays. The loaded lidocaine released rapidly in phosphate buffer saline and was found to be stable for 3 weeks.

(4) Hollow microneedles: The structure and mechanical principles of HMNs are similar to those of hypodermic needles with a channel; the centre of the needle tubes are hollow and there are holes at the needle tips. The compounds flow through the cavity of the needle, actuated by pressure equipment. The flow speed of compounds stored in the needle into the body can be completely controlled by specially designed devices, increasing personalized design for different drug delivery needs. Based on previous research, HMNs are used for high molecular weight compounds such as proteins, vaccines, and oligonucleotides [19].

Drug transport via microneedles

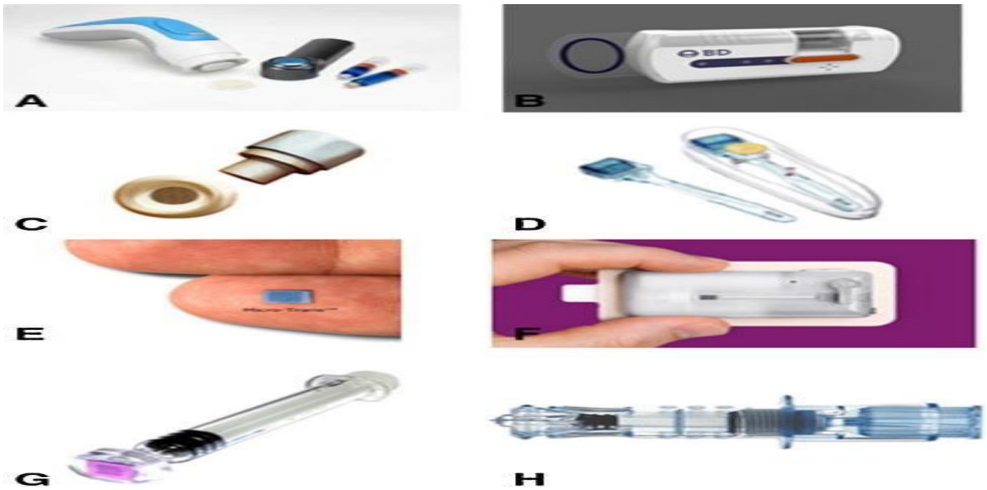
Drug delivery through microneedles can occur via two main pathways i.e. poke with patch or coat and poke method. In case of poke with patch technique pores are firstly made on the skin by microneedles and then after the removal of micro needles drug is applied on the skin. Where as in case in coat and poke method the microneedle surface is coated with the drug and then these microneedles is applied on the skin. So, drug transfer will occur via the needles surface. With the development of polymeric microneedles, a third approach was further developed in which drugs can be encapsulated in the polymeric matrix and released from the polymer upon application on the skin. Encapsulation of drugs within the polymeric core has an advantage of a higher drug loading as compared to other systems developed so far. Hence encapsulation of drugs within the microneedles has received most attention from transdermal drug delivery scientists in the past few years. Among all these approaches, coated microneedles are the most attractive mode for the delivering a rapid bolus consisting of high molecular weight molecules into the skin and can be considered similar to a simple band- aid like system for self-administration.

Insulin delivery via microneedles:

Insulin delivery is essential for controlling blood sugar levels in patients who have type 1 diabetes mellitus. Delivery of insulin through the SC (Subcutaneous) route by hypodermic injection is associated with infection risk, phobia of the needle, and unbearable pain. So, to overcome these problems, nowadays, insulin delivery is being researched through polymeric MN, which is the most fundamental approach for the delivery of insulin transdermally. Besides these, Lee (2017) and their associated research members developed a two-layer dissolvable polymeric MN, composed of CMC (Carboxymethylcellulose) and gelatin for insulin delivery.In that study, they also showed 85.7% relative bioavailability and 95.6% pharmacological availability of insulin delivered through polymeric MN.

Another research group from the University of North Carolina at Chapel Hill and North Carolina State University, Raleigh, United States fabricated MN patch with H202- responsive polymeric vesicles by using the polymers PEG (polyethylene glycol) and polystyrene for glucose-facilitated insulin delivery. They also proved that this MN patch could regulate Glucose level successfully with decreased risk of hyperglycaemia.

More recently, Chen's (2018) research team developed MN array with enzyme-free polymeric elements for transdermal insulin delivery [20].



Current microneedle devices. (A) Microstructured Transdermal System, (B) Microinfusor, (C) Macroflux®, (D) MTS Roller™, (E) Micro-trans™, (F) h-patch™, (G) MicronJet, (H) Intanza® [21]

Conclusion and outlook:

Microneedles, whether in the form of a patch or an array, have been identified as a viable carrier for the successful transdermal administration of a wide range of macromolecular medications. Several studies have found that microneedles are the best carriers for increasing drug absorption deep into the circulation system and offering a painless, effective, and safe method for drug delivery.

Currently, there are roughly 23 active and 39 completed National Institutes of Health (NIH) research studies involving MNs for the treatment of a number of ailments, including type 1 diabetes, psoriatic plaques, and topical anesthetics. The majority of studies make use of readily

accessible hollow MN infusion systems, and a few studies have looked at the efficacy of employing polymeric MN for protein drug delivery. For example, a Phase I trial examining the safety and immunogenicity of dissolvable MN for the delivery of H1N1, H3N2, and B seasonal influenza virus vaccine strains has been published (ClinicalTrials.gov identifier: NCT02438423). Recent studies have modified the initial MN methodology to a more patient-centered one, which offers a promising precedent for the future. To go to the next phase, we believe that legal questions and concerns from patients should be prioritized as we assess what stands between our existing MN expertise and the availability of an MN product for the patient. Human considerations such as safety, efficacy, and usefulness appear to be crucial. There are now multiple studies involving human volunteers, both with placebo MNs and drug-loaded MNs. These investigations served as the foundation for a preliminary short-term safety profile, with positive results thus far.

The second stage to evaluate is the long-term efficacy of MN application, both from an intermittent and recurring standpoint, such as when assessing the amount of dosages required for administering insulin over a prolonged time. According to studies on the administration of insulin via microneedles, blood glucose levels were much lower for a longer period of time as compared to subcutaneous injection. However, more research is required for improved patient efficacy and safety.

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CLINICAL IMPACTS OF NITISINONE IN ALKAPTONURIA

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Abstract:

Alkaptonuria (OMIM 203500), a rare autosomal recessive disorder caused by mutations in the HGD gene and deficiency of homogentisate 1,2 dioxygenase, is characterized by ochronosis, arthritis, and daily excretion of gram quantities of homogentisic acid (HGA) [20, 21]. Nitisinone, an inhibitor of the enzyme 4-hydroxyphenylpyruvate dioxygenase, can drastically reduce urinary excretion of HGA in individuals with alkaptonuria [1]. Homogentisate dioxygenase (HGD) is the enzyme involved in metabolism of tyrosine, which is characterized by the presence of dark ochronotic pigment in the connective tissue that is formed, due to high levels of circulating homogentisic acid [2]. HGA accumulates in multiple body parts and initializes tissue damage. Clinical manifestations such as pigmentation of the skin areas and joint destruction result in ochronosis [3]. This article focuses on the action, impacts and potential safety risks associated with nitisinone (NTBC) treatment for patients with alkaptonuria (AKU).

Keywords: alkaptonuria (AKU), nitisinone (NTBC); Homogentisate dioxygenase (HGD); hypertyrosinemia; keratopathy; adverse events;

Introduction:

Alkaptonuria, an autosomal recessive severe multisystemic disorder with an incidence of about one in a million live births, is caused by mutations in the HGD gene, which codes for a critical liver enzyme called homogentisate 1,2-dioxygenase. This enzyme in the tyrosine degradation pathway converts homogentisic acid (HGA) to maleyl acetoacetic acid [1,18]; a lack of this enzyme leads in excessive urine excretion of HGA and buildup of this compound in affected individuals' tissue. Standing causes the urine to darken due to the oxidation of HGA to benzoquinones, which form melanin-like polymers. These substances preferentially bind to connective tissue, resulting in ochronosis, which is characterized by darkening of ear cartilage, sclera, and bone, arthritis and joint degeneration, and heart valve disease. The use of vitamin C to boost HGA breakdown has not been proven [1]. Osteoarthritis and subsequent joint ochronosis seem to be an inevitable result of AKU, which causes severe disability and suffering at the height of maturity as a result of early joint and spine degeneration [17]. Patients with homogentisic aciduria, which typically presents as darkening of the urine upon standing, can be seen. Other symptoms that are connected, such ochronosis (pigmentation) of collagenous tissue and severe ochronotic osteoarthropathy [19] in the vertebral column and weight-bearing joints, have delayed presentations, typically beginning around the age of 30. Ochronosis can affect the heart valves, skin, ears, eyes, and ears. Fractures as well as black kidney and prostate stones are occasionally discovered [2].

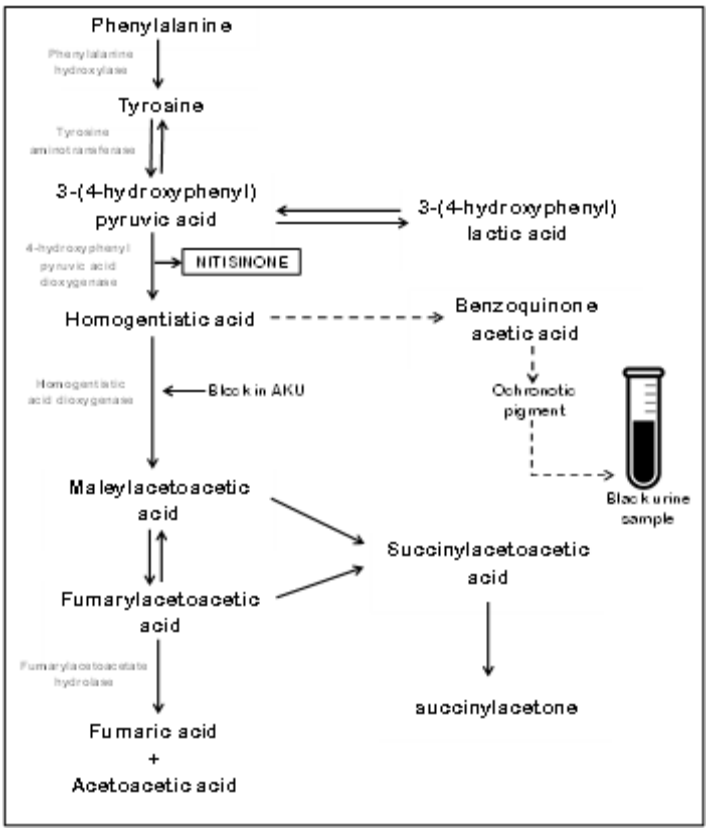

Fig. 1: While nitisinone inhibits 4-hydroxyphenylpyruvic acid dioxygenase and prevents synthesis of HGA, metabolic block in alkaptonuria (AKU) affects homogentisic acid dioxygenase (HGD) and causes its accumulation. Ochronotic pigment is created in connective tissue from a byproduct of benzoquinone acetic acid. Homogentisic aciduria (urine turns black when standing up due to the presence of HGA) and ochronosis (dark pigmentation of the ear, eye, and joints) are the main clinical manifestations of AKU

Aside from the accumulation of HGA (homogentisic acid), there is a suggestion that undiscovered proteins may be involved as potential causal elements in the deposition of ochronotic pigment, both within and outside cells. AKU has been classified as a secondary (AA) amyloidosis because to the presence of serum amyloid A (SAA) and serum amyloid P (SAP). Amyloidosis is a disease condition in which normally soluble proteins are transformed into insoluble fibrillar aggregates known as amyloid. These amyloid deposits, which can form throughout the body or in specific organs, are linked to a variety of illnesses. Amyloidoses are progressive disorders with a lag period, similar to the progressive character of AKU, which is also seen in other rheumatic joint diseases with secondary amyloidosis [16].

Ochronosis and amyloidosis:

There is significant evidence that AKU amyloid is tightly associated with the ochronotic pigment HGA-melanin. This suggests that HGA polymer may be involved in amyloid deposition, or that these two types of deposits, which are both resistant to various chemical and enzymatic treatments (detergent, acid, alkali, and protease), may share structural similarities. The significant co-localization of HGA-melanin and amyloid suggests that oxidised HGA pigment

may play a role in the development of amyloid aggregates, indicating a link between HGA oxidation and amyloid deposition [16].

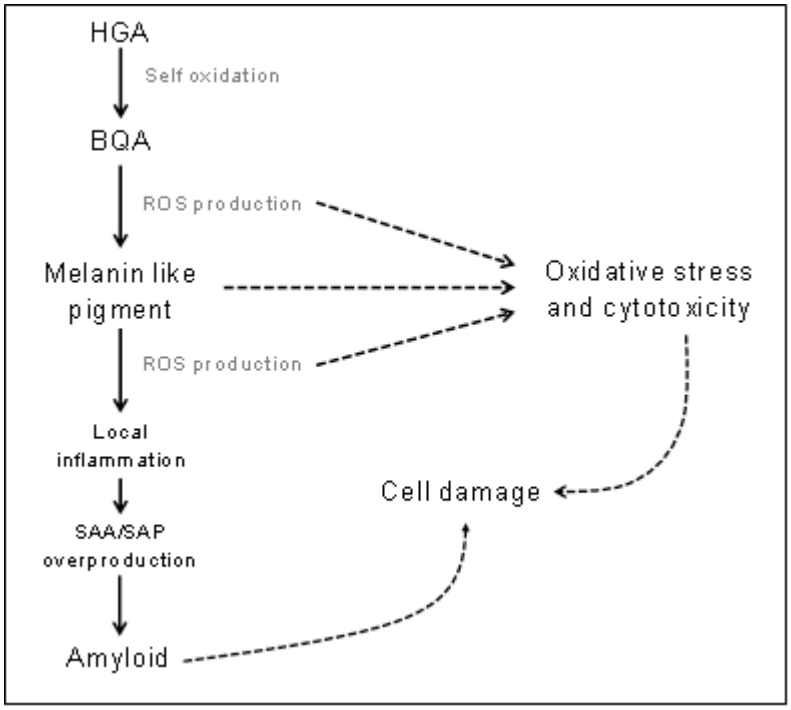

Fig. 2: In Alkaptonuria (AKU), a hypothesis suggests that the formation of ochronotic pigment and amyloid is linked to HGA and its auto-oxidation product BQA. Both the production process of these compounds and the resulting melanin-like ochronotic pigment contribute to oxidative imbalance, leading to cellular damage. This repetitive oxidative stress triggers a chronic inflammatory state, characterized by inadequate defense responses and abnormal production of amyloid-forming proteins, ultimately leading to secondary amyloid deposition. Amyloid, along with its intermediate forms, is also harmful to cells and promotes oxidative stress. Interestingly, similar to physiological melanogenesis, amyloid may be produced in an attempt to counteract the cytotoxicity of melanin or potentially serve as a scaffold for its synthesis. This suggests a complex interplay between HGA, ochronotic pigment, amyloid, and oxidative stress in AKU. In most ethnic groups, the prevalence of alkaptonuria (AKU), an uncommon hereditary condition, ranges from one in 250,000 to one in 1,000,000. It is more prevalent in some nations, such as Slovakia, Jordan, and India. With a recent map showing 1,233 individuals worldwide, the AKU Society has been instrumental in finding new AKU patients. Specific genetic changes in the HGD gene, which is found on chromosome 3, are the root cause of AKU. Due to the identification of more extensive gene deletions, mutation study for AKU often involves DNA sequencing and other cutting-edge methods. With 212 known HGD gene variations, the HGD Mutation Database has been created to gather mutation data from over 530 AKU patients. The ApreciseKUre database also gathers clinical and biochemical patient data [2].

The dietary condition of individuals affected by alkaptonuria

Historically, clinical therapy of AKU suggested limiting dietary protein consumption, particularly in children. Researchers investigated the potential benefits of protein and anti-inflammatory nutrients such as vitamin C, selenium, and zinc by examining their statistical

correlation with the AKU severity score index (AKUSSI), a validated measure of illness progression stratified by age. Results showed that approximately 50% of AKU patients have had some kind of protein restriction at some point in their lives. AKU patients have lower mid-upper arm circumference, grip strength, BMI, total calories, and protein intake, as well as a larger proportion of body fat, as compared to national data. As a result, they meet the criteria for "clinically undernourished" under ESPEN recommendations. The severity of their disease varies throughout their lives. However, there is no statistical evidence of a link between protein intake (expressed as a percentage of recommended nutrient intake or grammes per kilogramme) or anti-inflammatory nutrient consumption, such as high-dose vitamin C supplements, and disease severity when measured using the validated AKUSSI score [15].

Nitisinone: Nitisinone (2-(2-nitro-4-(trifluoromethyl)benzoyl) cyclohexane-1,3-dione) is a medication that has been used for treating children with Tyrosinaemia type I (TT1) for more than 20 years. It can lessen the production of HGA by acting as an inhibitor of the enzyme 4-hydroxyphenyl pyruvic acid [5]. Based on this behavior, it is reasonable to assume that treating AKU patients with nitisinone may lessen the disorder's effects, particularly the degradation of joints and the ochronosis of, say, the eye that occurs with chronic disease. Indeed, a number of recent studies have demonstrated that long-term administration of 10 mg of nitisinone daily for up to 4 years did slow the onset of AKU-related symptoms and stop or even reverse ochronotic pigmentation. In September 2020, the European Medicines Agency (EMA) granted official approval for the use of nitisinone (sold under the brand name OrfadinR) at a daily dosage of 10 mg as a treatment for adult patients with Alkaptonuria (AKU), based on the findings from the SONIA-2 study [3].

Adverse events included the passing of kidney stones, the recognition of symptoms related to aortic stenosis, and elevation of liver transaminase levels [1]. Nitisinone-induced tyrosinemia causes reversible dendritiform corneal keratopathy. In the NAC, there were three cases of keratopathy, a prevalence of 5%, all in the first 3 years after service commissioning, and it is likely that dietetic management enabled effective management of the sTYR and mitigated the risk of keratopathy [7]. Indeed, several recent trials have shown that long term treatment with 10 mg nitisinone daily for up to 4 years did slow the progression of AKU-related symptoms and even stopped or reversed ochronotic pigmentation. This was confirmed in a bigger research, SONIA-2, which compared nitisinone 10 mg daily to no treatment in 138 AKU patients from the UK, France, and Slovakia. Nitisinone at this dose reduced urine HGA by 99%, and the increase in AKU symptom score after 4 years was statistically lower in the nitisinone-treated patients. The subjects' average age at the onset of nitisinone treatment was 48 years. Despite the fact that nitisinone was well tolerated, there was a greater incidence of infections and eye-related complications.

Mechanism of action:

Nitisinone inhibits 4-hydroxyphenylpyruvate dioxygenase (HPPD), the second step in the tyrosine degradation pathway before the defective FAH enzyme produces HT-1 NTBC reduces the buildup of hazardous metabolites of the tyrosine breakdown pathway by blocking tyrosine

metabolism upstream of FAH. HPPD also functions as an upstream enzyme for homogentisate oxidase, another enzyme in the tyrosine breakdown pathway that is defective in alkaptonuria. In alkaptonuria, blocking HPPD with nitisinone prior to the defective step catalysed by homogentisate oxidase will avoid homogentisic acid buildup [25].

Route of administration

Nitisinone is approved for oral use via pill capsules or oral suspension. Nitisinone, while effective in treating certain conditions, is associated with several potential adverse effects that should be taken into consideration. These adverse reactions can be categorized into various domains:

Adverse metabolic reactions:

1. Elevated Levels of Tyrosine: Nitisinone treatment can lead to an increase in tyrosine levels in the body, which may have downstream metabolic implications.
2. Seizures: In some cases, nitisinone use has been associated with an increased risk of seizures, which is a serious neurological concern.

Adverse blood reactions:

1. Leukopenia: Nitisinone can cause a decrease in white blood cell count, a condition known as leukopenia, potentially weakening the immune system.
2. Granulocytopenia: This adverse reaction involves a significant reduction in granulocytes, a type of white blood cell, which can further impair immune function.
3. Thrombocytopenia: Nitisinone use has been linked to lower platelet counts, increasing the risk of bleeding and clotting issues.

Adverse ocular reactions:

1. Conjunctivitis: Some individuals taking nitisinone may experience inflammation of the conjunctiva, leading to symptoms like redness and discomfort in the eyes.
2. Eye pain: Ocular pain is another potential side effect, causing discomfort and distress.
3. Corneal opacities: Nitisinone can contribute to the development of corneal opacities, which may affect vision.
4. Photophobia: Increased sensitivity to light, known as photophobia, can be a consequence of nitisinone use.
5. Keratitis: This condition involves inflammation of the cornea and can result in vision disturbances and discomfort.
6. Cataracts: Long-term nitisinone use has been associated with an increased risk of cataract formation, potentially leading to impaired vision.

Adverse dermatological reactions:

1. Epistaxis: Nitisinone use may lead to nosebleeds or epistaxis due to its effects on blood clotting.
2. Exfoliative dermatitis: Exfoliative dermatitis is a severe skin condition characterized by widespread skin peeling and inflammation.
3. Pruritus: Itchiness or pruritus of the skin can occur as a result of nitisinone treatment.
4. Dry skin: Some individuals may experience dryness and discomfort of the skin.

5. Maculopapular Rash: Nitisinone use has been linked to the development of a rash characterized by small, raised red bumps.
6. Alopecia: Hair loss or alopecia may occur as a side effect of nitisinone, affecting both scalp and body hair. It's crucial for individuals prescribed nitisinone to be aware of these potential adverse effects and to promptly report any unusual symptoms to their healthcare provider. Additionally, regular monitoring may be necessary to manage and mitigate these side effects during treatment [23].

Impacts of Nitisinone:

Nitisinone is a well-tolerated medication, and the first side effect to be noted was the observation of corneal lesions developing in rats given the medication. This observation also led to the discovery of the medication for the treatment of HT1 (hypertyrosinemia type-1). The reduction of tyrosine in the diet can avoid hypertyrosinemia, which is probably one of the causes of these deposits. When dietary therapy is not given, nitisinone biochemically converts the enzymatic deficiency from HT1 to tyrosinemia type 3, causing high tyrosine concentrations up to 1,500 mmol/L (normal, 40-90 mmol/L). Patients with poor diet compliance have reported transient ocular symptoms as irritation, corneal erosion, and photophobia.

A longitudinal study, however, revealed that these opacities did not progress despite occasionally high plasma tyrosine concentrations. It is challenging to establish a level for tyrosine concentration that might be associated with corneal lesions because there does not appear to be an exact correlation between the plasma tyrosine levels and the development of the ocular symptoms [9].

Tyrosine concentrations:

A 36-month randomised clinical trial involving 40 individuals was started in 2005. Measures of musculoskeletal function served as secondary criteria, with hip total range of motion acting as the major outcome parameter. This study consistently showed a 95% reduction in HGA in urine and plasma over the course of three years biochemically. Primary and secondary criteria did not demonstrate a clinical benefit from the drug. Throughout the investigation, the mean plasma tyrosine in the untreated group stayed at 60 M. Patients in the nitisinone group had consistent, roughly ten-fold increases in plasma tyrosine (individual values ranged from 332 µM to 1528 µM; patient averages ranged from 670 µM to 826 µM) [5]. The effectiveness of giving nitisinone at a dosage sufficient to lower urine HGA excretion was investigated in an open-label, single-center trial. The mean plasma tyrosine level in this study was 68±18 (SD) µmol/L at baseline on a typical diet (normal, 35–90 µmol/L), however these levels increased quickly following nitisinone treatment. Patients 4 and 5 showed elevated plasma tyrosine concentrations to 407 and 250 µmol/L, respectively, despite having only 5 days of nitisinone at 0.35 mg bid. Seven patients received 1.05 mg bid of nitisinone, and their mean plasma tyrosine levels were 760±181 µmol/L. The plasma tyrosine levels of all 5 remaining patients decreased throughout the final week of nitisinone medication while on a diet limited to less than 40 g of protein per day, with decreases ranging from 52 to 216µ mol/L. On the protein-restricted diet, the mean plasma tyrosine concentration was 603±114 mol/L while it was 755±167 mol/L on a typical diet

[1]. In a different investigation, the tyrosine levels in tissues taken from NTBC-treated AKU mice were measured in order to gauge the severity of NTBC-induced hypertyrosinaemia. In order to ensure the safe use of NTBC in AKU, this work offers, for the first time, experimental verification of the severity of acquired tyrosinosis connected to NTBC. Tyrosinosis is likely present from the earliest embryonic stages in HT3, which displays a comparable deficiency to NTBC biochemically. HT3 often has neurological problems, despite reports of asymptomatic patients.25 In our research, NTBC increased the amount of tyrosine in the brains of AKU mice by roughly 8 times. Increased urine 3-methoxytyramine concentrations in mice with NTBC-induced hypertyrosinemia in the context of AKU suggest a shift in the peripheral catecholamine metabolism. NTBC-induced hypertyrosinaemia in people was not linked to depressed mood [8].

Nitisinone-induced keratopathy:

An adverse consequence of NTBC therapy is an increase in plasma tyrosine levels similar to tyrosinemia type 2, a disorder that also results in painful palmoplantar hyperkeratosis, conjunctivitis, and herpetic-like corneal ulcers. A 21-year-old woman who had been diagnosed with alkaptonuria was started on a low dose of NTBC (0.5 mg daily), and 4 days later she noticed some slight eye pain and sporadic visual blurring. Her complaints of sporadic visual blurring and eye discomfort persisted without getting worse; an optometry assessment found no abnormalities other than minor hypermetropia. After 7 months, the NTBC dosage was increased to 1.5 mg per day. She noticed noticeably more eye irritation, inflammation, and photophobia after 10 months of medication. After stopping NTBC, symptoms subsided over the course of a week, and NTBC was resumed. When the symptoms quickly returned, a second ophthalmology check revealed corneal crystalline deposits. Tyrosine crystals and symptoms disappeared a few days after the end of NTBC. Tyrosine levels and ophthalmic pathology in NTBC-treated alkaptonuria could not be directly linked, but this still provides extremely strong evidence for a potential nitisinone effect on the eyes [14].

Adverse Events:

1. One patient experienced vomiting and pain in his right side. There were both right and left hydroureter, hydronephrosis, and multiple black kidney stones that were passing. Throughout these incidents, the patient continued to receive nitisinone medication.
2. A patient whose AST (aspartate aminotransferase) serum levels were 31 U/L and 34 U/L, respectively. Two days after starting nitisinone (0.35 mg bid), his AST was 49 U/L and his ALT (alanine aminotransferase) was 46 U/L. His ALT was 95 U/L and AST was 75 U/L at day 5. With this level of ALT, the patient had to be removed from the therapy due to an adverse event. Serum liver function levels reached their highest on day 8 (ALT 117 U/L, AST 94 U/L), and three weeks later, they reverted to normal.
3. One of the patients had an AST of 16 U/L and an ALT of 25 U/L when they started the nitisinone treatment. Serum liver enzyme levels remained low until day 32, when the ALT and AST reached extremely hazardous levels of 144 U/L and 58 U/L, respectively. As a result, nitisinone has to be stopped. Within a week of quitting the medicine, ALT levels were back to normal.[1]

4. A 21-year-old man experienced one adverse event, developing tyrosine keratopathy and a skin rash seven weeks after taking 2-mg of nitisinone on alternate days. The keratopathy, dermatitis, and ocular problems all disappeared after nitisinone use was stopped. With no return of the ocular or skin issues, nitisinone was resumed at a dose of 2 mg once a week. [4]

Discussion:

Our review elaborates different kinds of clinical impacts and possible safety risks that come with treatment of Nitisinone (NTBC) especially when treating patients with alkaptonuria. Various pieces of evidence from numerous clinical trials were collected to demonstrate this. Points include the impact on serum tyrosine concentrations, majorly causing hypertyrosinemia, as well as various serious adverse events recorded during clinical trials and studies on patients with alkaptonuria. The adverse events were aimed to shed light on the possible complications that may arise during nitisinone treatment, especially when dealing with alkaptonuria patients with underlying diseases or conditions that may intervene with the treatment effect.

Tyrosinemia, which may result in corneal keratopathy and/or corneal opacity in the eyes of AKU patients, is the most common adverse reaction following nitisinone treatment. Although keratopathy and symptoms can go away as quickly as two weeks after stopping nitisinone [10], since keratopathy can be quiet [11], continued use of the medication will likely necessitate dietary monitoring and routine slit-lamp exams. It has recently been demonstrated that excluding tyrosine/phenylalanine from the diet considerably lessened the tyrosinemia that nitisinone-induced in mice. Restricting phenylalanine alone was ineffective. Similar to this, protein restriction greatly decreased the amount of tyrosine in the blood of AKU patients [12].

The emergence of hypertyrosinemia-related neurological issues is another potential nitisinone adverse effect. This has been suggested because nitisinone inhibits 4-HPPD, a protein whose lack causes tyrosinemia type III, a condition that may be linked to neurological issues [13].

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HEART OF THE FUTURE: ADVANCEMENTS IN CARDIAC TISSUE ENGINEERING

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Abstract:

The human body's first functional organ to develop is the heart. The heart begins to beat and pump blood just a few weeks into pregnancy, and it does so continuously throughout life. The most cutting-edge areas of current research in the treatment of cardiac disease are stem cell therapy and tissue engineering. With the help of these technologies, therapy for acute ischemic myocardial injury and chronic, otherwise irreversible, cardiac failure are improving. Massive cardiac muscle loss is brought on by myocardial infarction (MI), which is still the world's leading cause of death. Cardiac tissue engineering tries to restore function to the infarcted heart by infusing cells, bioactive substances, and/or biomaterials, or to replace the infarcted tissues with synthetic heart muscles. In order to restore both the structure and the function of damaged b myocardium, cardiac tissue engineering aims to develop functional tissue constructions. Functional human tissue units that combine biological fidelity, high-throughput screening, and real-time physiological response monitoring are poised to revolutionize illness prediction and drug screening**.**

Abbrevations:

Cardiomyocytes (CMs), Endothelial cells (ECs), Smooth muscle cells (SMCs), Myocardial infarction (MI) , Embryonic stem cells (EmSCs), Adult stem cells (AdSCs), Human embryonic stem cells (hESCs) ,Induced pluripotent stem cells (iPSCs), Bone morphogenetic protein 4 (BMP4) ,Vascular endothelial growth factor (VEGF), Gold nanoparticles (GNPs), Carbon-based nanomaterials (CBNs)

Introduction:

To create tissue-like structures, the interdisciplinary discipline of tissue engineering combines cells, biomaterials, biochemical (growth factors, for example), physical (mechanical loading), and biological signals (Mhanna and Hasan, 2017). Tissue engineering seeks to develop biological replacements that can preserve, repair, or enhance the function of harmed tissues. Despite the fact that the first tissue-engineered skin goods appeared in the late 1970s and early 1980s, giving rise to modern tissue engineering, the term "tissue engineering" wasn't actually coined until 1987. Regenerative medicine and tissue engineering are frequently used synonymously. Regenerative medicine, on the other hand, refers to methods for assisting a patient's body in healing a damaged tissue in vivo, as opposed to tissue engineering, which often entails creating a tissue in vitro (Gálvez-Montón *et al.,* 2013)

Acute or chronic heart muscle damage has long been thought to be the turning point in one's health and the beginning of heart failure. The issue is that adult cardiac myocytes, which

make up the heart's muscle cells, cannot divide to replace damaged ones. Because of this, the heart cannot heal itself through any natural processes, despite having a small number of cardiac stem cells that are present. Instead, areas of the myocardium that are injured become scar tissue. Such scar tissue preserves the organ but prevents it from contracting. The ideal treatment intervention would simply replace produced scar tissue with functional heart muscle tissue or would prevent such scar development altogether (Alrefai *et al.*, 2022.)

Structure of the heart

The heart is a sophisticated organ that circulates 7000 liters of blood daily throughout the body. Its anatomy is exactly determined by this pumping function. The epicardium, myocardium, and endocardium are the three layers that make up the complex organ that is the heart. Numerous distinct cell types, including as cardiomyocytes (CMs), endothelial cells (ECs), smooth muscle cells (SMCs), epicardial cells, fibroblasts, neurons, and immunological cells, are found within these layers. The cardiac muscle cells, or CMs, are found only in the myocardium and are responsible for the mechanical contractile function of the heart. Only 25–35% of the heart's cells are made up of these (Brady *et al.*, 2023).

Need for cardiac tissue engineering

It is widely recognized that cardiovascular disease is a leading global cause of morbidity and mortality. Traditional medicinal and surgical treatments have been effective in curing many cardiovascular conditions, including coronary artery disease and valvular illnesses, but they have been less effective in treating myocardial damage (Alrefai *et al.*, 2002) Approximately 90% of novel medications fail during phase 1 clinical trials, despite lengthy and significant investments of time and money.

Three million people will die from myocardial infarction (MI) in 2021, making it one of the leading causes of mortality and disability worldwide. Most patients who survive require ongoing medical care for the rest of their lives, which frequently involves recurrent invasive cardiovascular procedures. Thrombus development in a coronary artery, which reduces myocardial perfusion and prolongs ischemia, is a common cause of MI. Massive cardiomyocyte (CM) necrosis and an infarcted region are the effects of this. (Gil-Cabrerizo *et al.*, 2023).

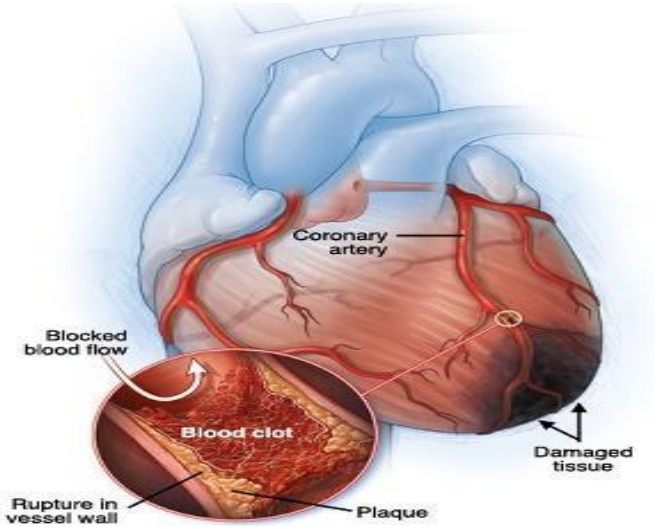


Fig. 1: Myocardial Infraction (MI)

Three essential factors need to be taken into account when creating tissue for use in cardiovascular therapy: sources of cells, scaffolds, and signaling elements

A. Scaffolds - A "scaffold" is a substitute that gives a new cellular microenvironment, which facilitates the development of new tissue, a structural platform. It permits cell motility, adhesion, differentiation, and organization, which can help supply soluble and bound biochemical substances (Alrefai *et al.,* 2002)

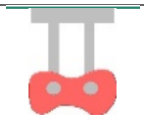
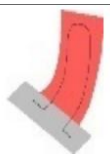
B. Cell source - Development of high-fidelity, three-dimensional (3D) cell culture methods for studying heart function, malfunction, and pharmacology in vitro are the core objectives of cardiac tissue engineering. They also aim to provide a ready supply of functioning tissues for therapeutic transplantation (Pomeroy *et al.,* 2021). Therefore, we need to understand the many cell types that are employed, where we can find them, how to harvest them, and whether we can cultivate them to produce additional cells. Because simply isolating the cells from the host tissue may not yield sufficient numbers. Therefore, we may have two cell cultures.

1) Autologous The word "auto" means "self" in the Greek language. So, whenever you see the term "autologous stem cells," it refers to the patient's own cells.
    - There is no chance of immunological rejection or disease transmission.
    - The availability is a drawback because it is scarce.
2) Allogeneic
    - The phrase "allogeneic stem cells" denotes the origin of the cells. That additional individual might be an adult or derive from the placenta or umbilical cord.
    - have greater availability and are less expensive
    - there is a risk of immune rejection and disease transmission
3) Xenogeneic
    - • using cells from non-human sources like pigsthere is a much higher risk of disease transmission and immune rejection.

C. Signaling elements

A new tissue's phenotype can be influenced by, or even directed by, signaling elements. They have direct and indirect impacts on cell metabolism, motility, and organization, and their use has been learned from signals detected during native tissue creation (Alrefai *et al.,* 2002). The objective of the new tissue graft will determine which cells are used to populate the scaffold. The majority of the mass of a tissue matrix will be created by the new cells, and they will also create the integrating connections with the native tissues that already exist (Jordan E Pomeroy, Abbigail Helfer *et al.,* 01 September 2021). While stem cells, and more recently adult stem cells, have emerged as the key players in the majority of new tissue replacement schemes, terminally differentiated cells have been used with varying degrees of success and have notable drawbacks when used in tissue engineering. Undifferentiated, pluripotent cells, stem cells can differentiate into a variety of cell lineages as well as self-renew indefinitely. From sources such as bone marrow, the placenta, tooth pulp, and adipose tissue, stem cells can be extracted (Alrefai *et al.,* 2022).

Tissue architecture

Tissue Architectures	Shape	Generation
Patches		Accumulation of cell layers on coated plates
Spheroids		Assembly of cell mixture and hydrogel
Ring		Condensation of hydrogel with cells in circular casting mold
Strip		Compaction of cells and hydrogel around two parallel wires
Cell Sheet		Seeding of cells onto coated film

Stem cell types used for cardiac repair

Undifferentiated, pluripotent cells, stem cells can differentiate into a variety of cell lineages as well as self-renew indefinitely. Adipocytes, bone marrow, placenta, tooth pulp, and other tissues can all yield stem cells. Autologous cells from the same individual have become the main focus of stem cell research in order to completely eliminate the possibility of allogeneic rejection.

Fetal cardiomyocytes- Fetal cardiomyocytes have an abundance of integration and regeneration capability.

EmSCs—EmSCs have a wide range of possibilities for cell differentiation into all three embryonic germ layers. Additionally, in vitro production of whole cardiomyocytes has been accomplished.

Human umbilical cord blood-derived cells - Many non-hematopoietic stem cells seen in human cord blood exhibit lower levels of class II human leukocyte antigens and don't seem to elicit an immunological response, reducing the risk of rejection.

AdSCs - Adult stem cells AdSCs have been proposed as a source for heart repair. MSCs, hematopoietic stem cells, and EPCs are all present in varying numbers in adipose tissue.

Skeletal myoblasts- Skeletal myoblasts can be harvested by muscle biopsy from the individual and grafted to cardiac tissue (16).

Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) are two of the most promising stem cell sources for heart tissue. The growth of a highly uncommon population of adult cardiac progenitor cells (CPCs) or epicardial cells are additional possible cell types that could be employed to repair heart tissue (Gil-Cabrerizo *et al.*, 2023).

Microenvironment for stem cells

The stem cell niche contains stem cell subpopulations that are crucial regulators for the preservation of stemness and tissue repair after injury. The surrounding cells in the stem-cell niche include both stem and stromal cells. Additionally, signaling chemicals, matrix architecture, physical forces, oxygen tension, and other environmental elements are present. The biochemical and biophysical cues required for cell proliferation, apoptosis, and differentiation are provided by this highly specialized and complicated environment, which enables stem cell self-renewal and function (Kathleen M. Broughton, Mark A. Sussman, December 2020). The reliability with which synthetic milieu can provide appropriate microenvironmental cues to sustain proliferation, self-renewal, and differentiation is crucial to the long-term success of stem cell-based tissue engineering systems. Whether the objective is to preserve the stemness or to differentiate stem cells into mature lineages, well controlled microenvironmental conditions (both physico-mechanical and biological) are essential for producing the appropriate stem cell fate. The following elements are necessary for a microenvironment to enable stem cell proliferation and differentiation into cardiomyocytes: (a) textural and topographic features; (b) biological signals; and (c) stem cell sources (Ogle *et al.,* 2016).

Tissue engineering of stem-cell derived myocardium

At physiological conditions, the self-renewal rate of CMs in the adult human heart is thought to be 0.45% at age 75 and 1% each year at age 25 (*Gil-Cabrerizo et al., 2023*). Cardiac tissue engineering seeks to produce cardiac tissue constructions that can contract synchronously with the host heart muscle, have the required thickness, tissue compactness, density of functioning cardiac cells, and other desirable characteristics (Wang *et al.,* 2016).

Embryologic development of stem cells in the heart

The origins of the heart's cells may have an impact on how well the organ works, suggesting that cells for tissue engineering and regenerative therapy should be carefully chosen. During the embryonic stage of the heart's development, mesodermal precursors give rise to the myocardial cells of the adult heart. The first and second heart fields, which develop into cardiomyocytes, and the subsequent growth of the four-chambered heart from a linear heart tube are orchestrated by signaling pathways (Montgomery *et al.,* 2014).

Differentiation of stem cells into cardiomyocytes

Human pluripotent stem cells generate cardiomyocytes in a specific developmental lineage during mesoderm induction. Adding recombinant human activin A and bone morphogenetic protein 4 (BMP4) to human EmSC differentiation methods causes the cardiac mesoderm to mimic the fundamentals of embryonic development (Novakovic *et al.*, 2023).

Cardiac purity after differentiation

Following differentiation, there are a number of ways to increase the yield and purity of the tissue sample. Centrifugation using a Percoll density gradient was one suggested approach. Another tactic was to cultivate cells in a lactate-rich media, which would kill or eliminate any cells that lacked enough mitochondria to survive (typically, these are noncardiac cells). 100% purity is challenging to attain. (Gil-Cabrerizo *et al.*, 2023).

Cell injection and cell sheet grafting

A. Cell injection

In recent years, research into cell-based techniques for heart tissue engineering and revascularization has increased. To replace the injured cells and enhance heart function, fresh cells are directly injected into the infarction site during in situ cellular transplantation therapy. After being administered to infarcted areas, it is hoped that those cells may aid in reestablishing the vascular structure and support the formation and remodeling of new blood vessels. Cell treatment, however, has had mixed results due to the challenges in regulating the size, position, and destiny of the implanted cells. Lack of a suitable extracellular environment to aid in the retention of transplanted cells and the structural and biomechanical integration of the restored region with the host tissue presents another hurdle for cell therapy (Wang *et al.*, 2016).

B. Cell sheet grafting

A multilayered, three-dimensional (3D) construct is created using cell sheet technology by stacking two-dimensional (2D) monolayer cell sheets. This method has the benefit of enabling the delivery of a significant number of cells with preserved cell-cell contact and potentially with cell-deposited ECM. After transplantation, cell sheet grafting has the ability to significantly increase the engraftment to host tissue and increase the efficiency of cell (Wang *et al.*, 2016).

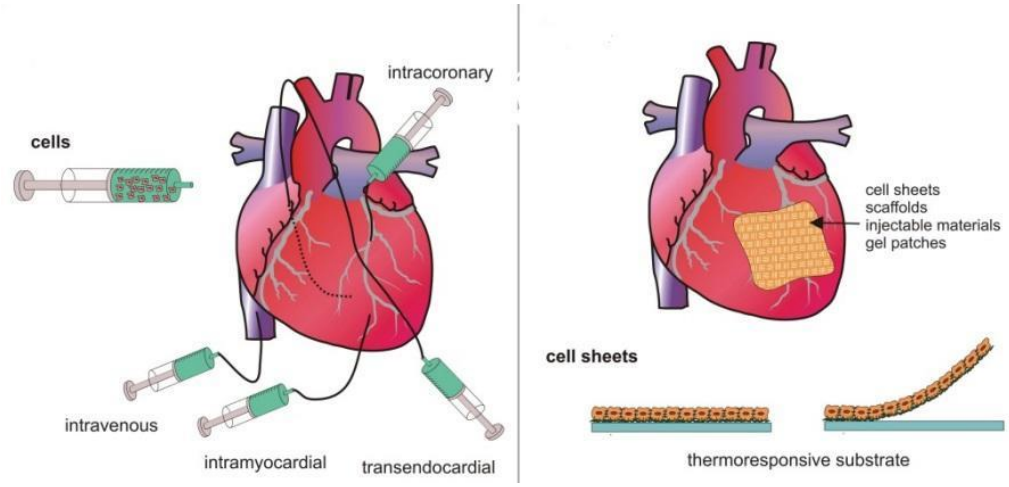


Fig. 2: Process of cell injection and cell sheet grafting

Vascularization

Forming blood vessels into a tissue to increase the amount of oxygen and nutrients it receives is a process known as vascularization. Each myofiber in native heart tissue is situated between two capillaries, which have an average spacing of 20 m between them and a diameter of 7 m. Strategies to encourage blood vessel creation, such as 1) cell tri-culture, 2) application of growth factors and peptides, and 3) building of novel proangiogenic scaffolds, have been

inspired by the need to provide sufficient oxygen and nutrients to created tissues. Microscale cardiac organoids performed better when fibroblasts, endothelial cells, and cardiomyocytes were grown in succession rather than simultaneously (Wanjare *et al.,* 2019). It has also been possible to start pre-vascular networks by sandwiching cardiac cell sheets between endothelial cells (ECs). The host vessels were connected to by these vascular structures, which promoted better vascularization. Angiogenic growth factors, including vascular endothelial growth factor (VEGF), can be introduced into biomaterials for tissue engineering as an alternative to cell tri-culture. The most common delivery methods today include physical and covalent immobilization, soluble factors, microparticles, and delivery systems. It takes the release of several growth factors from microparticles to produce a stable vasculature. An efficient way to create vascularized myocardium in vivo is to seed cells in chambers that have been subcutaneously implanted around an arteriovenous loop (Montgomery *et al.,* 2014).

Biomaterial scaffolds for cardiac tissue engineering

For cardiac tissue engineering, a wide range of materials have been taken into consideration. Decellularized tissues, synthetic materials, and materials produced from living things are all distinct categories.

Natural Materials

- **Collagen** - The most prevalent structural proteins in the ECM of mammalian myocardium are collagen I and collagen III. The majority of the tensile strength is provided by collagen I fibrils, but collagen III added to collagen I has been found to increase tissue elasticity. Collagen can be employed as a hydrogel or as a porous scaffold, respectively (Montgomery *et al.,* 2014).
- **Gelatin**- As gelatin is made from chemically denatured collagen, it is less strong and deteriorates more quickly than collagen (Montgomery *et al.,* 2014). When it degrades, it has been observed to cause an unspecific inflammatory response. At first glance, this may seem like an undesirable consequence, but for some applications, it may have a good effect on angiogenesis.
- **Fibrin** - Fibrin is the end result of fibrinogen's pro-protein being broken down by thrombin. In the initial clotting cascade and for the process of early wound healing, fibrin deposition is essential to hemostasis (Montgomery *et al.,* 2014). Fibrin can be fabricated ex vivo as well as as an injectable gel, expanding the range of possible production methods.
- **Alginate**- The use of alginate scaffolds produced by the freeze-drying process in cardiac regeneration has been extensively studied. They prevented left ventricular dilatation when implanted in mice with infarcts and loaded with fetal cardiac cells (Wanjare *et al.,* 2019).

Synthetic materials

- **Polylactic acid and Polyglycolic acid** - A biocompatible, biodegradable, and FDA-approved polymer, polylactic acid breaks down into lactic acid (which is non-cytotoxic) and has been used extensively in patients, including as sutures. Additionally, a

thermoplastic, polyglycolic acid has been applied in healthcare settings and breaks down into non-toxic compounds.

- Polyurethanes- Polyurethanes are synthetic materials that are biocompatible and frequently employed in the biomedical industry. By altering their composition, their mechanical characteristics and biodegradability can be customized.
- Acrylate based materials -Although acrylate-based materials have not yet been extensively used in cardiac tissue engineering, interest in them is growing due to their adaptability to processing and the range of features they can produce (Wanjare *et al.,* 2019).
- Gold nanoparticles (GNPs)- GNPs, including gold nanospheres (GNSs), gold nanorods (GNRs), and gold nanowires (GNWs), have been shown to be promising nanomaterials in biomedical-related applications, from imaging to diagnostics and particularly in tissue engineering and regenerative medicine. This is due to their biocompatibility features, ease of fabrication process, and unique electrical characteristics.
- Carbon-based nanomaterials (CBNs)- Carbon-based nanomaterials (CBNs), also known as nanoscale carbon allotropes such as carbon nanotubes (CNTs), graphene-based nanosheets, carbon nanohorns, and carbon nanofibers, have long been renowned for their exceptional capabilities. This class of materials has attracted a lot of interest in tissue engineering applications because of their mechanical, topological, and electrical characteristics (Esmaeilia and Patino-Guerrero, 2022).

Products for cardiac tissue engineering getting toward the clinic

A medical product's quality cannot be compromised from an engineering or business perspective in order to cover any other cost over the whole product life cycle. The standards of the quality control should be set for tissue engineering constructs in order to reliably supply high-quality products and lower the additional costs related to the manufacturing process (Wang, *et al.,* 2016). Over the past 20 years, cellular treatments for heart repair have advanced in clinical studies. Researchers are considering the preclinical model design, the complex medical problems of the human patient population with heart failure, and potential advances to cardiac cellular treatments in the clinic despite the modest gains in functional results and potential advancements in clinical cardiac cellular treatments. The utilization of tissue engineering techniques and products is one option that could lead to better functional results. Tissue engineering-based preclinical models have shown varied degrees of development and success. Phase I clinical trials have been conducted on the majority of the hybrid cells plus scaffold products that have made it to the clinic (Broughton and Sussman, 2020).

The future of cardiac tissue engineering

The currently available treatments for vascular problems still have a number of limitations and complications despite being effective. These treatments include transplantation, surgical reconstruction, the use of mechanical and synthetic devices, and the administration of metabolic products. In order to regenerate damaged arteries, it is therefore preferable to design in vitro and in vivo biomimetic structures for specific target organs or tissues (Esmaeili *et al.,*

2022). The promising field of cardiac tissue engineering has the potential to significantly advance our knowledge of inherited and acquired heart conditions and to speed the creation of novel regenerative treatments for myocardial infraction and heart failure (Pomeroy *et al.*, 2021). Another key area of interest in the field of cardiac tissue engineering is the production of viable tissues for patients with heart failure following MI. By including cells, biomaterials, or a combination of the two within a cardiac patch or injection, this method seeks to repair myocardial lesions. Pediatric patients with univentricular physiology were the focus of one of the earliest tissue engineering-based cardiac clinical trials (Broughton and Sussman, 2020).

The cardiovascular community continues to struggle with the development of a reliable cellular therapy for the restoration of heart muscle. Researchers have shown that cellular therapies have the ability to thicken the free wall, decrease scar tissue, and enhance myocardial vascularization. To lessen external stress on the heart and boost endogenous healing, these adjustments are crucial (Kathleen and Sussman, 2020). Synergistic delivery of bioactive cell-based products with biomaterial scaffolds will probably be the next horizon in alleviating human heart disease, according to recent clinical investigations with injectable biomaterials (Pomeroy *et al.*, 2021). The field will advance concurrently with developments in pluripotent stem cell technologies that will allow synthetic cardiac tissues to include adult functionality and patient specificity. The effectiveness of cardiac tissue engineering and regeneration depends on establishing functional perfusion and structure in the constructs/repairing regions despite all the difficulties (Wang *et al.*, 2016). In addition, the development and adoption of tissue engineering standards is turning into a crucial step for facilitating the clinical translation of bench-to-bedside cardiac tissue engineering research.

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BITES AND STINGS: NAVIGATING THE PERILS OF VENOM POISONING

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Abstract:

Venom poisoning, also known as envenomation, is a medical condition resulting from the injection of venomous substances into the body through the bite, sting, or other mechanisms employed by certain animal species. Venoms are complex mixtures of bioactive molecules, including enzymes, peptides, proteins, and toxins, which are produced by various animals such as snakes, spiders, scorpions, insects, and marine creatures like jellyfish and cone snails. The severity of venom poisoning varies widely depending on factors such as the type and quantity of venom injected, the location of the bite or sting, the size and health of the victim, and the species responsible for the envenomation. Symptoms can range from localized pain, swelling, and redness to systemic effects such as nausea, vomiting, muscle weakness, difficulty breathing, and even death in extreme cases. Medical treatment for venom poisoning is specific to the type of venom and the animal involved. It often involves supportive care, pain management, and interventions to counteract the effects of the venom, such as antivenom administration. Venom-derived compounds have shown potential in fields such as pain management, cancer treatment, and neurological disorders due to their unique interactions with biological systems. venom poisoning is a multifaceted medical condition caused by the introduction of venomous substances into the body. While it poses significant health risks, advancements in medical science and research continue to enhance our understanding of venoms and their potential applications in medicine. In this book chapter we are discussing the various types of venom poisoning, prevention, diagnosis as well as treatments.

Keywords: Venom, Antivenom, Envenomation, Bioactive, Drugs.

Introduction to venomous creatures:

Over 220,000 species, accounting for approximately 15% of the entire spectrum of animal life on Earth, exhibit venomous traits. [1] Venom equips predators with a chemical armament surpassing physical strength in potency. Animal venoms are intricate and advanced combinations of bioactive elements, primarily composed of proteins and peptides.[2] The most extensively studied animal venoms likely originate from cone snails, spiders, scorpions, and snakes. The composition of the venoms from the first three categories is dominated by brief disulfide-rich peptides (ranging from 3 to 9 kDa) that incorporate the inhibitor cysteine knot (ICK) pattern. However, more substantial proteins, including enzymes, are also present. ICK peptides possess remarkable structural stability and primarily affect the nervous system, chiefly targeting membrane channels or neuronal receptors. The venom from spiders or cone snails has the potential to encompass thousands of distinct peptides, while a scorpion's venom may contain several hundred.[3] The substantial diversity of spider species (possibly exceeding 100,000)

further amplifies the variety of venoms. Venomous creatures have long captured the human imagination with their mysterious allure and potentially deadly consequences. From the serpentine elegance of snakes to the intricate webs of spiders, the enigmatic stings of scorpions, and the graceful yet dangerous dance of marine animals, these creatures have evolved unique ways to deliver venom that can incapacitate or even kill their prey.[4] This book chapter will provide an overview of the diverse array of venomous creatures that inhabit our planet, highlighting their significance in various ecosystems and the ways in which they interact with humans.

The composition of venoms:

Venoms are complex mixtures of various molecules produced by certain animals, typically for the purpose of defense, predation, or competition. These molecules often include proteins, peptides, enzymes, toxins, and other bioactive compounds. [5-6] The composition of venoms can vary widely between different species and even among individuals within the same species.

1. Proteins and Peptides:

Proteins and peptides are major components of venom and play a crucial role in the toxicity and effects of venoms produced by various animals. These bioactive molecules target different physiological systems in prey, predators, or competitors. Here are some common types of proteins and peptides found in venom.

1.1 Neurotoxins: Neurotoxins, a potent class of venom components, possess a remarkable ability to target the nervous system with precision.[7] These toxins interfere with critical nerve signaling processes, leading to a range of effects that can incapacitate prey or deter threats. While neurotoxins are notorious for their harmful effects, they also hold potential therapeutic applications.

Mechanism of action: Neurotoxins exert their effects by interfering with nerve impulses and neurotransmitter signalling. They target specific receptors or ion channels involved in nerve transmission, disrupting the normal flow of electrical signals along nerve cells. By binding to these molecular targets, neurotoxins can block or modulate nerve impulses, leading to paralysis, muscle weakness, and even respiratory failure.[8]

2. Hemotoxins: Hemotoxins, a class of toxins found in the venoms of many snakes, play a critical role in the venomous arsenal by targeting the circulatory system and blood components. These toxins have evolved to disrupt blood clotting mechanisms, damage blood vessels, and induce internal bleeding, leading to a range of effects that can be both deadly and fascinating.[9] Beyond their harmful effects, hemotoxins also offer potential therapeutic applications. Here's an exploration of the mechanism of action, effects, and therapeutic potential of hemotoxins found in venom.

Mechanism of action: Hemotoxins exert their effects by interfering with the normal processes of blood clotting, which are essential for maintaining hemostasis and preventing excessive bleeding. These toxins can affect various stages of the clotting cascade, including the activation of platelets and the conversion of fibrinogen to fibrin, a process that forms blood clots. By

disrupting these processes, hemotoxins prevent the formation of stable clots, leading to internal and external bleeding.

3. Cytotoxins: Cytotoxins, a group of potent venom components, are fascinating in their ability to disrupt cellular integrity and trigger a cascade of effects.[10] These toxins play a critical role in the immobilization of prey and defense against threats, offering insights into the complex interplay between venomous creatures and their environments. Here's a closer look at cytotoxins, their mechanisms, effects, clinical implications, and emerging therapeutic potential.

Mechanism of action: Cytotoxins primarily target cellular membranes, which act as barriers that maintain cellular integrity and function. Cytotoxins disrupt these membranes through various mechanisms, including pore formation, lipid peroxidation, and enzymatic degradation. By compromising the integrity of cell membranes, cytotoxins initiate a series of events that lead to cell lysis, inflammation, and tissue damage.

4. Cardiotoxins: Within the complex concoction of venomous mixtures, one group of potent toxins stands out for their chilling effects on the cardiovascular system: cardiotoxins.[11] These specialized toxins, often found in the venoms of certain snake species, specifically target the heart and surrounding structures, leading to a cascade of physiological disruptions. Cardiotoxins showcase the remarkable adaptations that venomous animals have developed to incapacitate and subdue their victims with lethal precision.

Mechanism of action: Cardiotoxins are a diverse group of proteins that can interfere with the normal functioning of the heart and cardiovascular system. They often interact with specific receptors, ion channels, or other molecular targets found in cardiac tissues. By disrupting the electrical signals that regulate heart contractions and altering the delicate balance of ions, cardiotoxins can induce arrhythmias, cardiac arrest, and even sudden death in their victims.[12]

Enzymes: Venom, the potent secretion produced by various venomous creatures, is a complex mixture of bioactive molecules designed to immobilize prey, deter threats, or serve other ecological purposes.[13] Enzymes, as integral components of venom, play a pivotal role in enhancing the effectiveness of venom by facilitating the delivery and amplification of toxic effects. These remarkable catalysts are not only responsible for the swift incapacitation of victims but also hold potential therapeutic applications. Here, we delve into the world of venomous enzymes, their mechanisms, effects, and promising medical implications.

Mechanism of action: Enzymes in venom, intricate molecular tools finely honed through evolution, possess a remarkable ability to disrupt cellular structures and physiological processes within the bodies of prey, predators, or rivals. These catalytic agents play a pivotal role in enhancing the effectiveness of venom, contributing to rapid immobilization, tissue damage, and overall toxicity. Understanding the mechanisms by which these enzymes operate offers a glimpse into the intricate strategies venomous creatures employ to ensure their survival [14].

Disruption of cellular integrity: Enzymes in venom primarily target cellular membranes, which act as the gatekeepers of cells, regulating what enters and exits. By disrupting these membranes, venom enzymes trigger a cascade of effects that compromise cell integrity and function.[15] For example:

1. **Phospholipases:** These enzymes break down the phospholipids that constitute cell membranes. This disruption leads to the formation of pores or holes, causing cellular leakage and promoting inflammation and tissue damage.
2. **Proteases:** Venom proteases can cleave proteins within cells and tissues. By degrading important molecules, these enzymes contribute to tissue damage, bleeding, and inflammation.
3. **Hydrolases:** Disrupt cell membranes and tissues, enhancing venom spread. Found in certain spider venoms.

Table 1: Enzymes and peptides in various types of venom:

Venom	Enzymes	Proteins
Snake	Phospholipase A2 L-Amino acid oxidase Hyaluronidases Acetylcholine esterase	Sarafotoxins Lipopolysaccharide Bradykinin potentiating or angiotensin- converting enzyme inhibitors Neurotensin Phyllolitorin Litorin Tryptophyllin
Spider	Phospholipase A2 L-Amino acid oxidase Antithrombins Hyaluronidases	Antimicrobial peptides (cytolytic or cationic peptides) Cysteine-rich peptides Cystine knot inhibitor Psalmopeotoxin I, II Huwentoxin I
Scorpion	Hyaluronidases Phospholipase A2 Metalloproteinases L-Amino acid	Ion channel (Na+, Ca2+, K+ and Cl-) toxins Non-disulfide-bridged peptides (NDBPs)
Marine animals	Phospholipase A2 Metalloproteinases Endopeptidases Hyaluronidases Proteases Ribonucleases Lipases Collagenases	Conotoxins Anemone Toxins Tachykinins Aurelin Neuropeptides Cnidoin Antimicrobial peptides Ovulins Porins Enkephalins

Amplication of toxic effects: Enzymes in venom work in synergy with other venom components, such as toxins, peptides, and proteins. By breaking down cellular barriers and proteins, enzymes create pathways for other venom components to access and affect target tissues more effectively. This amplification of toxic effects accelerates the incapacitation of prey or the deterrence of threats.[16]

Facilitating venom spread: Some venom enzymes, like hyaluronidases, degrade components of connective tissues, promoting the spread of venom through tissues. This enhanced diffusion allows venom to reach deeper layers and distant areas from the bite or sting site, increasing the overall impact of the venom [17].

Evolving adaptations: Different venomous creatures have developed unique venom compositions to suit their ecological niches and hunting strategies. As a result, the mechanisms of venom enzymes can vary widely between species. Snake venoms, for example, might contain a combination of phospholipases, metalloproteinases, and other enzymes that work in concert to immobilize prey [18].

Research and medical implications: Studying the mechanisms of venom enzymes not only sheds light on the intricacies of the natural world but also holds potential medical implications. Understanding how these enzymes interact with cellular structures can inspire the development of targeted drugs for various conditions. Venom enzymes might become valuable tools for drug delivery, cancer therapy, tissue engineering, and more. [19]

Therapeutic potential: While enzymes in venoms are notorious for their harmful effects on prey and threats, their unique properties and mechanisms of action have also captured the attention of researchers exploring potential therapeutic applications.[20] These enzymes, once feared as part of nature's arsenal, are now being considered as tools to address various medical challenges. Here's a glimpse into the promising therapeutic potential of enzymes found in venoms.

Targeted drug delivery: Enzymes' ability to disrupt cellular membranes has inspired the concept of using them as vehicles for targeted drug delivery. Researchers are investigating ways to attach therapeutic compounds to venom enzymes, effectively creating "nanocarriers" that can navigate through the body and selectively penetrate specific cells or tissues. This approach could revolutionize drug delivery by increasing the precision and effectiveness of treatments, minimizing side effects, and improving patient outcomes. [21]

A) **Cancer Therapy:** Enzymes in venoms, such as certain proteases and phospholipases, have shown potential for cancer therapy. These enzymes can specifically target cancer cells, exploiting their vulnerabilities and triggering cell death. By harnessing the properties of venom enzymes, researchers aim to develop innovative treatments that offer a more targeted approach to cancer therapy, minimizing damage to healthy cells [22].

B) **Anticoagulant Research:** Some snake venoms contain enzymes that interfere with blood clotting mechanisms. Understanding these enzymes' effects on coagulation pathways could lead to the development of novel anticoagulant drugs. These drugs could provide more

targeted and effective treatment options for conditions like deep vein thrombosis, where preventing excessive blood clotting is crucial [23].

C) **Tissue engineering ang regeneration:** Enzymes with matrix-modifying properties, such as metalloproteinases, have applications in tissue engineering and wound healing. By carefully manipulating these enzymes, researchers can encourage tissue regeneration, facilitate wound closure, and enhance the recovery process after injuries or surgeries [24].

D) **Immune modulation:** Venom enzymes might also have potential in modulating the immune response. By understanding how these enzymes interact with immune cells and signaling pathways, researchers could develop therapies that regulate immune reactions in conditions like autoimmune diseases and inflammatory disorders [25].

E) **Neurological Disorders:** Some venom enzymes could be explored for their effects on nerve signalling and neurological conditions. Understanding their interactions with neurotransmitter receptors could offer insights into developing treatments for disorders like epilepsy, chronic pain, and neurodegenerative diseases [26].

In-depth profiles of venomous snakes: Exploring neurotoxic, hemotoxic, and cytotoxic venoms

In snake venoms, a typical mixture includes 20 to >100 components, with the majority (>90%) being peptides and proteins. Predominant bioactivities involve neurotoxicity, haemotoxicity, and cytotoxicity, contingent on the snake species. Venom composition displays considerable variation between species, and even within the same species. Additional factors such as environmental conditions, age, gender, and the available prey type can also influence venom composition. Venomous snakes are among the most captivating and potentially dangerous creatures in the world. Their venomous adaptations have evolved to suit a variety of ecological roles, from paralyzing prey to deterring predators.[27]

Neurotoxic venoms are particularly potent, as they target the nervous system of the victim. These venoms contain specialized molecules that interfere with nerve signal transmission, leading to paralysis and often rapid death. Species such as cobras, kraits, and mambas are known for their neurotoxic venoms. When injected into their prey, neurotoxins can cause muscle weakness, paralysis, and even respiratory failure. While these venoms are highly effective for subduing prey, they can also pose significant dangers to humans if bitten.[28]

Hemotoxic venoms are designed to cause damage to blood cells and tissues. These venoms contain enzymes and toxins that disrupt blood clotting, leading to internal bleeding and tissue destruction. Many vipers, such as pit vipers and rattlesnakes, possess hemotoxic venoms. When these snakes bite, their venom can lead to swelling, tissue necrosis, and a range of systemic effects. The hemotoxic components are particularly efficient at breaking down tissues, aiding in the snake's ability to digest its prey.[29]

Cytotoxic venoms primarily target and break down cells at the site of the bite. These venoms contain enzymes that cause cell membranes to rupture, resulting in tissue damage and necrosis. Some vipers and pit vipers possess cytotoxic venoms. While these venoms may not

lead to rapid paralysis like neurotoxins, they can cause extensive local damage, resulting in severe pain, swelling, and the potential for secondary infections.[30]

Each type of venom has evolved to suit the specific hunting and defense strategies of the snake species. Neurotoxic venoms are particularly effective for quickly immobilizing prey, while hemotoxic and cytotoxic venoms aid in the digestion of prey and fending off threats. Some snakes possess a combination of venom types, allowing them to adapt to a broader range of ecological niches.

Understanding the different types of snake venoms is crucial for minimizing risks when encountering these creatures. Different antivenom treatments are tailored to counteract the effects of specific venom types. When bitten, seeking immediate medical attention and identifying the snake species responsible for the bite are essential steps in ensuring effective treatment.

Table 2: Different snake venoms, their primary functions and potential drug application:

Snake Venom	Primary Function	Potential Drug Application
Neurotoxic Venom	Target and disrupt the nervous system	Neuromuscular disorder, Pain Management
Hemotoxic Venom	Cause damage to blood vessels and blood cells	Blood Thinners, Clot related disorders
Cytotoxic Venom	Destroy Cells and Tissues	Cancer Treatment, wound Healing
Cardiotoxic Venom	Affect the Cardiovascular System	Heart Disease, Arrhythmias
Coagulopathic Venom	Impair Blood Clotting Mechanism	Blood Clotting disorders
Mytotoxic Venom	Damage muscle tissues	Muscle related condition
Nephrotoxic Venom	Affect the kidney and urinary systems	Kidney disease, Renal disfunction
Enzymatic Component	Catalyze specific biochemical reactions	Enzyme replacement therapy
Anticoagulant Venom	Inhibit blood clotting factor	Anticoagulant medications
Anti-inflammatory Venom	Reduce inflammation and pain	Inflammatory disorder, Pain Relief
Analgesic Venom	Provide Pain Relief	Pain Management, Analgesics
Antimicrobial Venom	Exhibit activity against microbes	Antibacterial, Antifungal Agents
Anticancer Venom	Show potential in inhibiting cancer cell growth	Cancer treatments, Target therapies
Angiogenesis inhibiting venom	Supress formation of new blood cells	Anti-angiogenic therapies
Immunomodulatory venom	Modulate the immune response	Immune related disorders

Exploring spider venoms: Unveiling neurotoxins, necrotic agents and silk proteins:

Table 3: Examples of different spider venoms, their functions and potential drug application

Spider Venom	Function and Effects	Potential Drug Application
Latrodectus spp. (Widow Spiders)	Neurotoxic venom causing pain, muscle cramps, and other symptoms	Pain relief, muscle relaxants
Phoneutria spp. (Brazilian Wandering Spiders)	Neurotoxic venom leading to pain, paralysis, and priaprism	Pain management, erectile dysfunction treatment
Loxosceles spp. (Recluse Spiders)	Cytotoxic venoms causing tissue necrosis and systemic effects	Pain Management, antispasmodics
Theraposidae spp. (Tarantulas)	Mild venom causing local pain and swelling	Analgesics, anti-inflammatory agents
Phoneutria spp. (Brazilian Wandering Spiders)	Neurotoxic venom leading to pain, paralysis, and priaprism	Pain management, erectile dysfunction treatment
Atrax spp. (Funnel Web Spiders)	Neurotoxic venom including pain, muscle spasms, and respiratory issues	Pain management and antispamodics
Lycosa spp. (Wolf Spiders)	Mild venom causing localized pain and redness	Analgesics and inflammatory agents

Spiders are intriguing creatures known for their intricate webs and, in some species, their potent venoms. Spider venoms are a diverse cocktail of molecules that serve various purposes, from immobilizing prey to protecting the spider itself.[31] In this section, we'll delve into the three main components of spider venoms: neurotoxins, necrotic agents, and silk proteins. Some spider species, like the infamous black widow, are equipped with neurotoxic venoms. These venoms contain specialized molecules that target the nervous system of their prey. Neurotoxins disrupt nerve signal transmission, leading to paralysis and immobilization. When injected into their prey, neurotoxins cause muscle weakness, twitching, and in severe cases, paralysis. These venoms are adapted for subduing a wide range of prey and are especially effective against insects. In contrast to the rapid effects of neurotoxins, other spider venoms, such as those of the brown recluse spider, contain necrotic agents.[32] These venoms cause tissue damage and cell death at the bite site. The necrotic properties of these venoms result in localized tissue necrosis, often leading to an open wound that may take a considerable amount of time to heal. Necrotic venoms aid in the spider's ability to digest its prey by breaking down tissues and enabling the spider to consume liquefied nutrients.

While silk isn't a venom in the traditional sense, it's an integral part of a spider's predatory arsenal. Silk proteins are produced by specialized glands in a spider's abdomen and are used to create webs for trapping prey, constructing shelters, and even capturing mates. Spider silk is incredibly strong and lightweight, making it an engineering marvel. Some spiders have evolved to produce silk with venomous properties, using it to immobilize or subdue struggling

prey ensnared in their webs. Different spider species have evolved unique venom compositions that suit their ecological roles. Neurotoxic venoms are tailored to rapidly paralyze and subdue prey, while necrotic venoms enable spiders to begin digestion before consuming their meals.[33] Silk proteins represent a multifunctional tool for building webs, capturing prey, and ensuring survival. While spider bites are relatively rare and fatalities are even rarer, understanding the types of venoms present in various spider species is essential for minimizing risks. Most spider bites cause mild reactions, such as localized pain and swelling, but a few species have venoms that can lead to more severe symptoms. Identifying spiders and understanding their behaviors can help mitigate potential encounters and ensure appropriate medical care if a bite occurs.[34]

The sting of scorpions: Unravelling different venom types and their effects on humans

Table 4: Examples of Scorpion venoms, their effects, functions, and potential drug applications:

Scorpion Venom	Function and effects	Potential Drug Application
Centruroides spp. (Bark Scorpion)	Neurotoxic venom causing pain, muscle twitching, and neurological symptoms	Pain Management, Muscle relaxants
Androctonus spp. (Deathstalkers)	Neurotoxic venom leading to pain, paralysis, and cardiovascular effecs	Pain Relief, Antihypertensive agents
Hottentotta spp. (Indian red scorpions)	Neurotoxic venom causing severe pain, muscle spasms, and nother effcts	Pain Mangement, muscle relaxants
Leiurus spp. (Deathstalker)	Neurotoxic venom including pain, paralysis and other systemic effects	Pain relief and antispamodics
Tityus spp. (Yellow fat-tail scorpions)	Venom causing local pain, inflammation and mild systemic effects	Pain Relief, anti-inflammatory agents
Opisthacanthus spp. (Desert hairy scorpions)	Venom with mild local effects and minimal systemic impact	Analgesics, anti-inflammatory agents

Scorpions, with their distinctive appearance and venomous stings, have intrigued and sometimes frightened humans for centuries. Their venoms are complex mixtures of molecules that vary across species and can have diverse effects on the human body.[35] Some scorpion species possess neurotoxic venoms that target the nervous system of their prey and potential threats. When injected into a victim, neurotoxins disrupt nerve signal transmission, leading to paralysis, muscle twitching, and difficulty breathing. These venoms can affect both the central nervous system and peripheral nerves, causing symptoms ranging from localized pain and numbness to more severe reactions like muscle paralysis and respiratory distress. While not as common as neurotoxic venoms, some scorpion species have hemotoxic venoms that affect blood cells and tissues. These venoms can cause blood clotting problems, leading to bleeding disorders, and may also damage blood vessels and surrounding tissues.[36]

Hemotoxic effects can result in pain, swelling, and tissue necrosis at the sting site. In severe cases, these venoms may cause systemic effects like organ damage and internal bleeding.

Cytotoxic venoms, found in certain scorpion species, are designed to break down cells at the site of the sting. These venoms contain enzymes and toxins that cause cell membranes to rupture, leading to tissue damage, inflammation, and necrosis. Cytotoxic venom effects can include local pain, swelling, and the potential for secondary infections due to damaged tissues. Scorpion venoms have evolved to suit the ecological roles of different species.[37] Neurotoxic venoms are adapted for quickly incapacitating prey or threats, while hemotoxic and cytotoxic venoms may assist in digesting prey and deterring predators. Each venom type reflects the specific niche and hunting strategies of the scorpion species. Although scorpion stings can be painful and uncomfortable, fatalities from scorpion envenomation are relatively rare, particularly in healthy adults. However, certain individuals, such as children, the elderly, or those with underlying health conditions, may be more vulnerable to severe reactions. It's important to seek medical attention if a scorpion sting occurs, especially if symptoms worsen or systemic effects are observed.[38]

Underwater dangers: Navigating venomous marine animals

The oceans are home to a vast array of creatures, many of which possess venomous adaptations that are both fascinating and potentially perilous to humans. In this section, we'll explore the underwater dangers posed by venomous marine animals, including jellyfish, cone snails, and other intriguing inhabitants of the deep.[39]

Jellyfish are graceful and captivating creatures, but their tentacles can deliver potent venomous stings. The tentacles are equipped with specialized cells called nematocysts, which contain venom-filled capsules. When touched, these cells can discharge venom, causing a range of reactions in humans, from mild discomfort and skin irritation to severe pain, swelling, and, in rare cases, systemic reactions. Some species, like the box jellyfish, have venoms that can lead to heart failure and death within minutes. Understanding jellyfish species, their habitats, and how to avoid encounters is crucial for staying safe in marine environments.

Cone snails, though less known than some other marine creatures, possess venomous harpoons that are ingeniously adapted for capturing prey. These snails use their venomous darts to immobilize fish, injecting a potent cocktail of toxins that paralyze their victims. Some cone snail venoms contain compounds with potential medical applications, such as pain relief and treatment for neurological disorders. However, their stings can be extremely painful and sometimes fatal to humans. Awareness of cone snail habitats and behaviors is essential to avoid accidental stings.[40]

Beyond jellyfish and cone snails, the ocean harbours numerous other venomous creatures. Sea anemones, while resembling plants, are predatory animals armed with venomous tentacles to capture and immobilize small fish and crustaceans. Lionfish, with their stunning appearance, possess venomous spines that serve as a deterrent to predators and as a means of capturing prey. Stonefish, known for their remarkable camouflage, have venomous spines that can cause excruciating pain, tissue necrosis, and even death in severe cases. Staying safe in marine environments requires a combination of awareness, caution, and understanding. Learning to recognize venomous marine species, understanding their behaviors, and adhering to best practices for avoiding encounters can greatly reduce the risk of stings or injuries. For individuals

who explore the oceans or engage in activities like snorkeling or diving, proper training, protective equipment, and knowledge of local marine life are crucial.[41]

Table 5: Examples of different marine animal venom, their primary functions and potential drug application

Marine Animal Venom	Effects and Primary Functions	Potential Drug Applications
Box Jellyfish	Neurotoxic venom causing intense pain and cardiovascular effects	Pain relief, antihypertensive agents
Cone Snail	Peptide-based venom targeting specific receptors, leading to paralysis or pain	Pain management, neuropathic pain treatment
Blue-Ringed Octopus	Neurotoxic venom causing paralysis and respiratory failure	Respiratory stimulants, pain management
Sea Anemone	Contains proteins that affect ion channels and cause local pain	Analgesics, anti-inflammatory agents
Stonefish	Venomous spines delivering potent toxin causing severe pain	Pain relief, anti-inflammatory agents
Sea Urchin	Venom causing local pain, redness, and inflammation	Analgesics, wound healing agents

7. Venom Based Approved Therapeutics: Several venom-derived compounds have been explored and approved for therapeutic use in various medical applications. These compounds, often derived from the venoms of snakes, spiders, and other venomous animals, have demonstrated potential in treating a range of conditions. Here are a few examples of venom-derived compounds that have been approved for therapeutic use or are in advanced stages of development:

1. **Capoten (Captopril):** Captopril, an angiotensin-converting enzyme (ACE) inhibitor used to treat hypertension and heart failure, was developed from a peptide found in the venom of the Brazilian lancehead viper (Bothrops jararaca).
2. **Byetta (Exenatide):** Exenatide, a medication used to treat type 2 diabetes, is a synthetic version of a peptide found in the venom of the Gila monster lizard (Heloderma suspectum). It works by increasing insulin secretion and decreasing glucagon release.
3. **Prialt (Ziconotide):** Ziconotide is a synthetic version of a peptide found in the venom of the cone snail Conus magus. It is used as an analgesic to manage severe chronic pain, particularly in cases where other treatments have been ineffective.
4. **Aggrastat (Tirofiban):** Tirofiban, used as an antiplatelet agent to prevent blood clot formation, was inspired by proteins found in the venom of the saw-scaled viper (Echis carinatus).
5. **Botox (Botulinium Toxin):** Botulinum toxin, derived from the bacteria Clostridium botulinum, is used in cosmetic and medical treatments to temporarily relax muscles and reduce the appearance of wrinkles. It also has therapeutic applications for conditions like migraines, muscle spasms, and certain neurological disorders.

6. **Angiomax (Bivalirudin):** Bivalirudin, a direct thrombin inhibitor used as an anticoagulant during certain medical procedures, was developed based on a protein found in the venom of the medicinal leech (Hirudo medicinalis).
7. **Eptifibatide (Integrilin):** Integrilin, an antiplatelet medication used to prevent blood clot formation, was developed from a peptide found in the venom of the southeastern pygmy rattlesnake (Sistrurus miliarius barbouri).
8. **Zinplava (Bezlotoxumab):** Bezlotoxumab is an antibody developed from the venom of a South African snake (Dendroaspis angusticeps). It is used to prevent recurrent Clostridium difficile infection in certain patients.

Epilogue: A venomous future

In conclusion, venom poisoning is a complex and potentially life-threatening condition that arises when individuals encounter venomous creatures such as snakes, spiders, or insects. The effects of venom can vary widely depending on the species and the specific toxins involved, but they often result in a range of symptoms, from local pain and swelling to systemic reactions affecting vital organs. Prompt recognition and appropriate medical intervention are crucial in managing venom poisoning cases, as they can significantly improve the chances of a successful outcome. Furthermore, ongoing research into venomous creatures and the development of effective antivenom treatments are essential for reducing the global burden of venom-related injuries and fatalities. Public education on venomous species and preventive measures also play a pivotal role in minimising the risks associated with venom poisoning. As we continue to advance our understanding of venom and the effects, we can strive for better prevention, treatment, and ultimately, the preservation of human lives.

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USE OF NANOTECHNOLOGY IN SMART PILLS FOR GASTROINTESTINAL DISEASE DIAGNOSTICS, TREATMENT AND SAMPLING

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Introduction:

Smart pills are the ingestible pills or capsules which is made up of nanoparticles which contains electronic or mechanical elements which can look into the gastrointestinal lining and help in gastrointestinal disease diagnose, treatment and even sampling. These smart pills are made with the help of nanotechnology. Nanotechnology is the technology which deals with engineering the system at the atomic and molecular level. As the characteristics of a material or element is very different as compared to the characteristics of materials comes in a nanoscale. At nanoscale, everything starts changing and we can use it for our benefits. Many techniques used to diagnose, treatment and sampling of gastro intestinal diseases are highly invasive and very painful and even expensive with repetitive visit and sittings with Dr is needed, which is very tedious and torturous to the patient. Along with it, it is observed that the heath care is facing a huge challenge due to body's inability to absorb the whole dosage of drug. Therefore, by the help of nanotechnology, we can architect smart pills in such a way, that can help in the target drug delivery along with biologics, where biologics are found to be hard to administer and can be done only by infusion methods which is again an invasive method for administration of drug. We can also architect capsule by putting camera in it with the help of nanotechnology and can-do capsule endoscopy which will ultimately help in diagnosis and detailed study of gastrointestinal tract and its related diseases. We can consider smart pill under nanomedicine i.e., nanotechnology and medicine. Some of the gastrointestinal diseases, which can be diagnosed are, gastroesophageal reflux (GERD), esophageal cancer or inflammatory bowel diseases (IBD), intestinal cancer and also irritable bowel syndrome. These nanomedicine helps majorly in the concept of original and purified personalized medicine. Though capsule endoscopy is successfully translated from lab to clinically used products, but still there are many different challenges which is being faced still now and it has to be overcome.

Smart pills for diagnosis

Huge efforts are been taken from many years to use smart pills for the diagnosis of gastrointestinal diseases and to know the cause of disease with detailed information about the gastrointestinal tract. The efforts have been taken to utilize many different technologies like auto fluorescent imaging, optical for taking a look inside the gastrointestinal tract and other technology such as sensors check physical changes taking place inside the GI tract like pressure, pH or chemical analyte. The pills which we will discuss now are in its early development along with good clinical efficacy but also faces many challenges which has to be solved.

Imaging

Capsule endoscope depends on the optical imaging of the mucous surface so that we can detect GL disease using visible wavelength of light. These capsules are powered by silver oxide battery and also has camera, light emitting diodes for I Illumination, microprocessor and telemetry to send images to external receiver via app or something else for later analysis. CEs may have different camera's frame rate to acquire images at a specific rate in a specific region of the GI tract such as fast rate of pictures in esophagus and slow rate in colon. For colon imaging They may have more than one camera and also have varying battery life that is from 0.5-15 h.

But along with such a wide array of benefits, it is observed that because this, devices do imaging using only visible wavelength, it restricts diagnosis to nonspecific changes in the mucosal surface of the tract. Along with this sometimes miniaturization also becomes a problem when we start adding other advance technologies along with Camera to make the imaging clearer and easier to diagnose the disease. Some of the examples of this is Pill cam capsule Endoscope (Medtronic) and smart pill (Medtronic) refer in Fig 1. Some capsules also integrate imaging technology such as optical coherence tomography (17) where this capsule overcomes the limitation of Pillcam (1,2).

1. Auto fluorescence imaging

This technique illuminates the tissue with short wavelength of light almost 380-500 nm to excite endogenous or exogenous fluorophores and emits light at longer wavelength at almost 490-590 nm. This principle allows differentiation between malignant and benign. Tissue because malignant tissue emits lower light as compared to that of benign tissue. This imaging technology is widely used with conventional endoscopy (3).

2. Endoscopic ultrasonography

This technology uses ultrasound for imaging of GI tract and is clinically accepted along with routine clinical use. The main goal of this technique is to miniaturization (4,5,6,7).

a) The Khuri-Yakub group in Stanford University of USA are attempting to make a capsule with multi- element ring array, so that it can generate 360 ° image of GI tract with the ultrasound frequency of 5MHz, which has greater penetration depth & lower spatial resolution leading to giving us images of the organ located beyond the GI tract (6). But this technology is still in its infancy and needs to be capsulated neatly and tested in- vivo.

b) Sonophil project of UK is focusing on producing single-element transducers which will operate at frequency as high as 30 MHz. This technology, focuses mainly on spatial resolution on the sacrifices of penetration depth for greater resolution of structural and tissue composition. But is has sufficient penetration for the imaging of intestinal wall with sufficient resolution that can differentiate between the two layers of the intestine or bowel (5).

3. X-Ray imaging

The c-scan system is one of the X-say imaging smart pill which uses a short-lived radio isotope ^{191}Os which has a half-life of 15.4 days. This gives a transmurally images of the Colon after the ingestion of iodine-based Contrast agent (8,9). The emitted x-rays are divided into three rotating beams which helps to give radial image of colon wall. A number of in vivo clinical trials

have been conducted with the C-Scan system which demonstrates its safety (8) along with its clinical potential (10,11) and further validation and direct comparison of the system is required with the standard colonoscopy (8).

4. Optical coherence tomography

This is the technique used for sub-mucosal imaging which is capable of high spatial resolution as compared to conventional histological analysis of excised biopsy time. This technique does its imaging by measuring the time delay and degree of back- reflected light from the tissue sample on which the optical beam is scanned (12). This technique has high sensitivity & specificity which is sufficient to differentiate between Crohn's disease and ulcerative colitis, which gives us the indication that this technique can be used for the diagnosis of the inflammatory intestinal bowel disease (13).

Physical sensing

1. Pressure sensing

The changes in the GI tract can indicate that body is going through some pathological conditions. One of the best examples of this is bowel motility changes, which is mainly associated with GI conditions such as abdominal discomfort, pain and altered bowel habits. Current methods for this include endoscopic insertion of manometer which is invasive method. This limitation has forced to make many capsules which will help to check and measure pressure in small intestine. Smart Pill is the capsule now available which is the only wireless devices which has capability of pressure sensing, along with pH sensing and temperature sensing. This Smart Pill can sense temperature from range of 25 -49^0 C along with pH range of0.05- 9.0 and also a single pressure sensor with range of 0-46 KPA with the sensitivity of $^+$ and – 0.650 K Pa (14).

2. Temperature sensing

Human body can regulate its body temperature when it is exposed to different environment or even when increased physical activity. Usually, this pill is not for the use of diagnostic purpose of any disease but is mostly used to detect the internal body temperature of the athletes (15).

Some of the pills available are CorTemp (USA) (16), Vitalsense (USA) (17), e-Celsius (USA) (18) and My Temp (Netherlands) (19).

3. pH sensing

The earliest ingestible capsules developed in 1965 was for pH sensing named Heidelberg p n sensing capsule. It has the RF antenna which is p n sensitive which is encased in an inert plastic shell of mmm diameter and from length. This sensor shows huge accuracy which is + or – 0.5 p n along with a pH range of 1 to 9 p H. The first approved capsule by FDA at 2006 is the smart Pill capsule from Medtronic, which helps to identify the pH change in the GI tract from range of 0.05 to 9.0 with accuracy (20).

One of the commercially available pH sensing capsules is wireless BRAVO capsule (Medtronic) which helps us to detect gastroesophageal reflux disease by doing the recording of esophageal pH for up to 96 h during its temporary attachment to esophageal wall. (21)

Chemical and biological sensing

Even a small change in the chemical and biological composition of the GI tract can let us know the various different pathological disease presence due to the difference in the chemical and biological make up. Therefore, capsules are made to sense this chemical & biological changes and will can diagnose the disease before larger damage to the body.

1. Haemoglobin detection

Conventional WLI-CE is used for identifying and detecting the blood present in the stomach, but it was not able to differentiate between past and active bleedings in the stomach. A colorimetric detection pill was produced which uses a hue -saturation light colour detention method on the blood cells which is selectively channeled into the measurement chamber, which has white LEDS for illumination, colour sensor and also an adsorptive colour sensitive film which changes its colour from white to red in the presence of blood. This can detect Hb with as low as concentration 2.375 mg/ ml which is less than the Hb found in the stomach breeding (22). Many different pills are generated, some of them are one of the pills developed by Nemiroski which is wireless, battery powered pill which detects active blood in stomach through the intravenous injection of fluorescein which emits light in presence of blood (23). Another pill is HemoPill from Germany (24).

Fig. 1: Diagnostic Capsules: A) Pill Cam Capsule Endoscope (Medtronic) B) SmartPill C) Sonocap (Sonopill project, UK) D) Optical Coherence Tomography pill (OCT)(USA) E) Gas sensing Capsule F) Autofluorescence Imaging Capsule (UK) G) X-Ray Imaging Capsule H) HemoPill Optical Blood Sensing Capsule

2. Gaseous biomarkers

Smart pills are also created to detect gaseous biomarkers which is associated with the metabolic activity of the microbiome. Kalantar Zadeh *et al.* developed a wireless electronic pill which successfully demonstrates the real-time measurements of hydrogen, carbon dioxide and oxygen gas levels in the GI tract. This capsule is of 9.8 mm diameter and 26 mm long which also

has a non-specific, semiconducting metal oxide sensor which is highly responsive to all oxidizing gases under aerobic and anaerobic conditions that are calibrated to detect hydrogen, carbon dioxide and oxygen. Intestinal gas enters into the capsule through a semi-permeable membrane which contains embedded nanoparticles that excludes the water. The pill shows the capability of some localization due to monitoring of changing oxygen concentration along the GI tract. Also, measuring the changing levels of hydrogen provides a means of understanding of the GI tract's microbial fermentation. This pill can be used to diagnose irritable bowel syndrome (25,26).

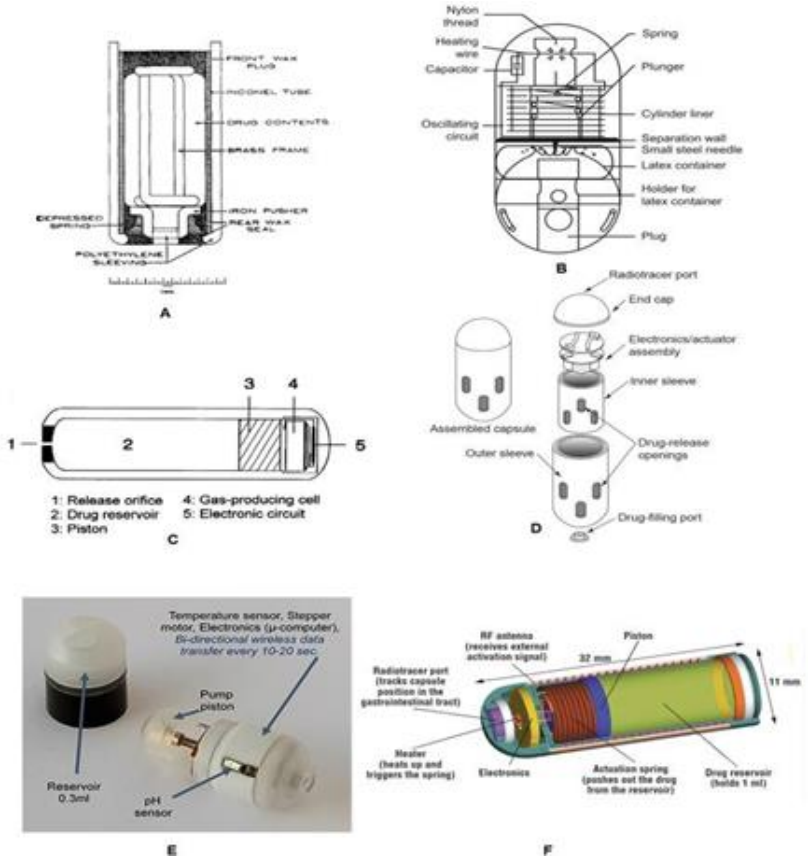

Fig. 2: Smart pills for drug and formulation- A) A 16 mm x 8mm stainless steel cylinder sealed with wax that utilizes inductive heating of the steel cylinder to melt the wax, releasing an internal spring which pushes the drug into the GI tract. Drug absorption studies in canine models are been done. B) The 28mm x 12mm HF- capsule utilizes 1ml latex balloon as an internal drug reservoir made from latex balloon which releases drug when punctured by needle which is released when a nylon thread melted when triggered due to external signal) A capsule that emptied the drug reservoir because of the motion of piston due to an electrolytic gas producing cell. D) The Intellisite drug delivery capsule which delivers drug passively over a time. E) The Intellicap drug delivery capsule which contains 0.3 ml of drug reservoir with stepper motor and integrated pH sensor, where this pH sensor is used for detection of the pill's location inside the GI tract. F) Enterion capsule has 1ml of drug reservoir along with a separate reservoir for radioactive marker to allow tracking of pill by gamma scintigraphy. The drug is emptied when small heater behind the piston is powered by coil within the capsule which is stimulated by induction

Smart pills for therapy

Smart pills are used for administration or to support various therapies, such as the targeted delivery of small molecules and drugs and transepithelial delivery of biologics. The design of the smart pills varies depending upon its application.

1. Drug delivery and drug formulation

The use of smart pills for drug formulation studies helps us to get detailed and realistic information about the specific drug formulation which is absorbed from some key areas of the GI tract. This would help in the reduction of delays observed during Phase 1 clinical trials, where this delay is due to the trial and error approach used for the multiple drug formulations (27) and also removes the need for invasive perfusion based techniques (28,29) one of the major advantage of smart pill is the target delivery of the drug molecules because otherwise these drugs are consumed in a generalized amount and its metabolism inside our body creates problem for our body by damaging the liver and kidneys. The use of drug-device combinations, more specifically drug delivery capsules along with integrated sensing or imaging capabilities, helps in detecting and treating diseases in GI tissue, this allows targeted therapeutics and lower side effects in patients. These therapeutic smart pills most probably operate on the same principle by either diffusing or expelling the active pharmaceutical ingredient (API) from an internal reservoir of the pill into the surrounding GI fluid after detecting some form of an environmental or external trigger. Smart pills that utilize diffusion to release the API are known as using passive drug delivery mechanisms, while those that expel out the API from the capsule are known as using an active drug delivery mechanism (30,31). Irrespective of passive or active delivery, most smart pills are currently passively propelled through the GI tract due to peristaltic forces and then eventually is excreted out with little control of their position or velocity at any given time. Due to this passive locomotion, drug delivery with these capsules inside the small intestine becomes challenging due to the fast transit time and lack of retention of pill or its anchoring mechanism.

2. Passive drug delivery

As the drug delivery in passive delivery takes place through diffusion, the drug delivery rate is dependent on the diffusion rate of the drug in the environment.

a) One of the first passive pills to be widely used was the HF-Capsule (32,33) as shown in Fig. 2B, the capsule size is 28 mm x 12 mm along with an internal drug reservoir which is made from a latex balloon with a maximum volume of 1 ml. An oscillating circuit present in the other half which is water-resistant absorbs energy from an externally supplied signal, which leads to wire heat up and melt the nylon thread which holds a spring attached to a needle under tension. As soon as the thread melts, the spring is released which results the needle to displace and puncture the latex balloon reservoir (34). But this pill has one of the disadvantages due to the balloon mechanism, that is incomplete emptying of drug and also it was challenging to fill balloons with powders (35)

b) Another smart pill which is used for the study of pharmacokinetics is the InteliSite Companion Capsule (USA), which is shown in Fig. 2D, the idea and need for developing

this pill was due to the challenges faced by the usage of HF capsule (36). An earlier version of the InteliSite device is made up of a size of 10 mmx35 mm along with internal drug reservoir of 0.8 ml. The capsule contents are released when external RF signal triggers it, which leads to heating up of integrated resistors. The thermal energy is dissipated to shape memory alloy metal wires generating a mechanical force which causes the inner sleeve of the capsule to rotate leading to aligning the ports located on the inner and outer sleeves and releasing the drug via diffusion. Afterwards InteliSite devices changed the mode of operation (37); as the earlier pill this capsule also contained an outer sleeve and inner sleeve where the inner sleeve was removable and was mounted on a spring. The inner sleeve was held in the place by a cap. The heating of these wires is again triggered by an external RF source which releases the cap and the inner cage-like sleeve containing the drug and then drug is also released. One of the disadvantages of the InteliSite capsule is that it is susceptible to gradual and preactivation leakage of liquid formulations of the drug due to a poor seal between the inner and outer sleeves (38).

3. Active drug delivery

a. Smith Kline and French developed, a stainless-steel cylinder sealed with wax with the size of 16 mm x 8 mm which was used to assess salyicate absorption in the different parts of a canine GI tract (39). A cross-sectional view of the capsule is shown in Fig. 2A. During the experiment, the canine was placed inside a large induction heating coil, which ultimately lead to heating of the ingested metal capsule and melt the wax, which releases a mechanical spring and expels the drug contents into the GI tract. Some of the disadvantage of this pill is the high temperature and long activation time needed and also that the subject must be placed within a bulky inductive heating coil for the effective use of pill.

b. Another early demonstration of smart pills capable of active delivery were created by Groening, Germany, an example of which is shown in Fig. 2C. This capsules usually first expelle out the drug via pistons pushed by gas created by electrolysis initiated by an external electrical signal (40,41,42). The final version of this capsule was created in 2007 with the size of capsule as 28 mm x 8.5 mm, which is capable of storing 0.17 ml of drug payload. However, the capsule was successful only on the benchtop experiments as it shows the potential.

c. One of the currently used capsules in the pharmaceutical industry is the Enterion capsule (3) as this helps in the assessment of drug absorption in the GI tract. This 32 mm long capsule, shown in Fig. 2F, contains an internal reservoir which is capable of storing up to 1 ml of the formulation. The reservoir is filled by the desired drug to be delivered into the 9 mm diameter port which is then afterwards sealed by using push on cap with silicone O- ring seal before ingestion. A separate sealed compartment is made to allow a radioactive marker to be emitted during passage through the GI tract so that the identification of the location of the capsule using gamma scintigraphy can be done. Drug release is triggered by switching oscillating magnetic field on a low MHz which then induces power in a tuned coil which is embedded within the capsule and this power ultimately heats a small heater rapidly in

another sealed compartment behind a piston. The heater increases the temperature rapidly which leads to breakage of a high tensile strength polymer filament which is otherwise used to restrain a coiled spring. Once the filament breaks, the spring pushes the piston forward, which ultimately leads to the emptying out of the drug due to increase in the pressure against the push-on cap which eventually gets removed and drug is released from the reservoir into the target tissue.

d. The Intellicap (Netherlands) can contain about a 0.3 ml internal drug in its reservoir with the size of the capsule as 27 mm x 11 mm plastic shell (43). In this capsule, the drug is forced out of the vents from the side of the capsule due to the actions of the internal stepper motor. The capsule has a level of flexibility which is not observed in other capsules in which various release profiles can be programmed, which then helps in the continuous release profiles of various speeds and also in intermittent release (single or dual burst profiles). The type of release profile is limited only by the 48-hour battery life and with the minimum release time of 10 min. This capsule can be operated either autonomously, or based on the input from the integrated temperature or pH sensors or a timed delay or remotely by an operator triggered RF signal. The pH sensor helps in the detection of position of capsule in the GI tract which then removes the need for integrating radioisotopes for tracking which is done in the Enterion capsule or external imaging technology. The Enterion and Intellicap are no longer commercially available. However, the SmartTab (USA) is a new commercial product which is currently undergoing trials that this actively deliver drugs using smart polymer mechanism in a smart pill.

Conclusion:

Therefore, we can conclude that, despite having such a huge clinical potential of these devices, smart pills are still in their infancy. Despite the intensive efforts taken for research activity in smart pills, many of them are yet to undergo the extensive in vivo testing which is necessary to demonstrate the diagnostic or therapeutic efficacy of these pills. The vision of autonomous smart pills which is capable of diagnosis with minimal clinical involvement along with the treatment is some way off. As these pills are so complex due to which has high cost and therefore denying wider patient benefit and make the devices more at risk of the failure. It is more probable that instead of one pill which has capability of all functions, a family of pills will be created, with their designs where each of it is optimized to perform a specific function, and work together or in sequence to ensure patient well-being. The solutions to the various problems and challenges faced by each type of smart pill can be overcome by intrinsically interdisciplinary, which requires expertise from electronic engineering, mechanical engineering, computer science, microengineering and materials science. Increasing smart pill functionality to incorporate and enhance navigation, multiple diagnostic or therapeutic use along with localization and the design constraints makes the smart pill build new challenges to ensure ease of ingestion and safety. For example, greater systems integration brings the risk of power consumption which will be especially challenging because batteries currently take up a large amount of space in capsule endoscopes. This requires new solutions to ensure that space is

available for other functions as well and due to this miniaturization of the pill becomes one of the huge challenges which has to be overcome.

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STEM CELL RESEARCH FOR THE TREATMENT OF OSTEOARTHRITIS OF KNEE JOINT

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Abstract:

Knee osteoarthritis (OA) is a degenerative joint disease characterized by the progressive deterioration of articular cartilage, leading to pain, stiffness, and reduced joint function. Traditional treatment options often provide only symptomatic relief and do not address the underlying causes of the disease. In recent years, stem cell therapy has emerged as a promising approach for the management of knee OA, offering the potential to promote tissue regeneration and slow disease progression. This abstract summarizes the current state of research on stem cell therapy for knee osteoarthritis, highlighting its mechanisms of action, clinical efficacy, and safety profile. It also touches upon the challenges and future directions in this evolving field of regenerative medicine. Stem cell therapy involves the use of mesenchymal stem cells (MSCs) derived from various sources, including bone marrow, adipose tissue, and umbilical cord blood. These multipotent cells possess the capacity to differentiate into chondrocytes, the cells responsible for cartilage formation and maintenance. Additionally, MSCs exert anti-inflammatory and immunomodulatory effects, which can help mitigate the chronic inflammation associated with OA. Clinical studies investigating the use of stem cell therapy for knee OA have shown promising results. Patients receiving stem cell treatments have reported improvements in pain relief, joint function, and quality of life. These outcomes are often sustained over an extended period, suggesting the potential for disease modification. Furthermore, stem cell therapy has demonstrated a favorable safety profile, with minimal adverse events reported in clinical trials. Despite the encouraging findings, challenges remain in optimizing the standardization and scalability of stem cell therapies, determining the ideal cell source, and refining the delivery methods. Additionally, long-term data on the durability of treatment effects are needed to establish the true therapeutic potential of stem cell therapy in knee OA. In conclusion, stem cell therapy holds great promise as a novel and effective treatment approach for knee osteoarthritis. This emerging field has the potential to revolutionize the management of OA by addressing the underlying pathology and providing long-lasting relief for patients. Continued research and clinical trials are essential to further elucidate the optimal protocols and long-term outcomes associated with stem cell therapy in knee OA.

Introduction:

Knee osteoarthritis (OA) is a debilitating degenerative joint disease that affects millions of individuals worldwide, primarily the elderly population. It is characterized by the gradual deterioration of articular cartilage, leading to pain, stiffness, and reduced joint function. Conventional treatments such as physical therapy, analgesics, and, in severe cases, surgical

interventions like knee replacement have been the standard of care. However, these options have limitations, and researchers have been exploring innovative approaches to address the root causes of OA and promote tissue regeneration. Stem cell therapy offers a novel and exciting approach to manage knee osteoarthritis. It leverages the regenerative potential of stem cells to repair damaged cartilage, reduce inflammation, and alleviate pain. This treatment modality has gained considerable attention in recent years due to its potential to revolutionize orthopedic care.

The science behind stem cell therapy (Guo, 2018)

Mesenchymal stem cells (MSCs), in particular, have shown significant promise in the field of knee OA treatment. These cells can be harvested from various sources, including bone marrow, adipose tissue, and umbilical cord tissue. Once isolated, they can be prepared and injected directly into the affected knee joint. The therapeutic potential of MSCs lies in their ability to modulate the inflammatory response within the joint, stimulate the production of chondrocytes (cartilage cells), and secrete bioactive molecules that promote tissue repair. This regenerative process aims to restore the damaged cartilage, improve joint function, and reduce pain, offering a more holistic approach to managing knee OA.

Clinical evidence and success stories (Wang, 2020)

Numerous clinical studies and trials have demonstrated the efficacy of stem cell therapy in knee OA management. A study published in the "Journal of Translational Medicine" in 2018 (Guo *et al.*) reported significant improvements in pain relief and knee function after MSC injection. Similarly, a meta-analysis published in the "Journal of Orthopaedic Surgery and Research" in 2020 (Wang *et al.*, 2020) confirmed the positive outcomes of MSC therapy in knee OA patients. Furthermore, anecdotal success stories abound, with patients reporting reduced pain and improved mobility following stem cell treatments. While more extensive research is needed to establish standardized protocols and long-term safety, these initial findings are undeniably promising.

Challenges and future directions:

Despite the potential benefits, stem cell therapy for knee osteoarthritis faces several challenges. These include refining the optimal cell source, dosage, and delivery methods, as well as ensuring the safety and long-term effectiveness of the treatment. Regulatory frameworks also need to be established to govern this emerging field.

In conclusion, stem cell therapy represents an exciting frontier in the treatment of knee osteoarthritis. As research continues to evolve, it holds the promise of offering a more effective and lasting solution for individuals suffering from this debilitating condition. While further studies and clinical trials are necessary to establish its efficacy conclusively, the initial results and the potential for regenerative medicine to transform orthopedic care are indeed encouraging. Knee osteoarthritis (OA) is the most common joint disease, with a systemically metabolic previous joint injury are well-known risk factors for knee OA. Obesity and body mass index (BMI) has a strong association with knee OA incidence. Abnormal joint loading can contribute to the process of obesity-induced OA. Obesity is also associated with hand OA, indicating that, along with abnormal biomechanical loading, other mechanisms could link these two conditions.

Nowadays, obesity is considered as a chronic inflammatory state, and adipose tissue, which was previously regarded as a simply passive energy store, has been recognized as a major endocrine organ. It can produce adipokines such as leptin, resistin, visfatin, chemerin, and also inflammatory cytokines such as tumor necrosis factor (TNF)-a, interleukin-1 (IL-1)-b and IL-66, which not only affect the neighboring cells but also impact target tissues' pathological processes.

Adipokines and inflammatory cytokines are involved in cartilage inflammatory mechanisms that may be on the casual way in knee OA (Chang, 2018). Intra-articular, intramuscular and intermuscular adipose tissues ac-count for a small proportion. The adipose tissues have been recognized as active endocrine organ or a main energy store of the body, which may play a role in the onset and progression of knee OA. This review addresses recent findings about the involvement of adipose tissue in knee OA, focusing on the roles of systemic and local adipose tissue in the pathogenesis of knee (Chang, 2018). Osteoarthritis (OA) is one of the most ubiquitous joint disorders; the prevalence of symptomatic hip and/or knee OA is ~242 million worldwide with conditions ranked as the 11th highest contributors to global disability. Decreased patient quality of life. Knee OA (KOA) demonstrates higher incidences compared to other joints, with a lifetime risk of ~45%, increasing to 60.5% amongst obese patients. Prevalence increases with each decade of life, with annual incidences highest between the ages of 55–65 years further exacerbated by endogenous and exogenous risk factors. OA results from degradation of the osteo chondral unit composed of: articular cartilage; calcified cartilage; subchondral and trabecular bone, which synergistically support functional loading. Throughout OA progression, degradative enzymes are overexpressed, including matrix metalloproteinases (MMPs), which degrade both matrix and non-matrix proteins. Chondrocyte senescence and reduced cartilage elasticity alters the tissue microenvironment impairing regeneration. Morphological changes in the subchondral bone include cartilage surface fibrillation and synovial fluid thickening, accompanied by progressive synovitis and osteophyte formations (Doyle, 2020).

Alternatively, cellular regenerative therapies, including mesenchymal stem cells (MSCs) and cell- derived products (such as platelet-rich plasma have shown therapeutic promise. BMSCs have generated considerable interest as a candidate for cell-based therapy because they are easy to isolate and expand in vitro, possess immune regulatory properties, and secrete a variety of trophic mediators. In addition to promising treatment of graft-versus-host disease, preclinical studies have shown beneficial effects of BMSCs on neurological disorders. BMSCs facilitate nerve regeneration, improve diabetic neuropathy, and multiple sclerosis and help functional recovery after stroke in rat models of the infra orbital nerve (ION) (Guo, 2011) degradation, synovial inflammation and bone erosions.

Mechanisms of action of adipose tissue on knee OA

The responsible mechanisms for the association between adi-pose tissue and knee OA appear to be multifactorial. Biomechanical factors, especially abnormal mechanical loading, play important roles in the initiation and development of knee OA. A recent study reported that weight and fat- free mass were significantly associated with knee OA after adjustment for metabolic factors, suggesting that mechanical stress is most important for knee OA. In obese

individuals, body adipose tissue mass may stress articular cartilage beyond biological capabilities, leading to cartilage damage. Additionally, IPFP is situated in close proximity to the patellar ligament around the joint and may reduce the instability and injury to the joint (Lee, 2019).

demonstrated that the weight-matched mice fed with saturated fatty acids (FAs) or u- 6 polyunsaturated FAs had significantly higher injury-induced OA severity than the mice fed with low-fat and u-3 polyunsaturated FAs diets, and OA severity was significantly associated with dietary fatty acid contents and serum adipokine levels, but not with bodyweight54. In a cross-sectional study, there was a positive and in-dependent association between high serum cholesterol levels and radiologic OA. Taken together, these data indicate that abnormal lipid profiles may explain the association between obesity, metabolic syndrome and OA (Cheng- Fong Chen, 2021).

We previously reported a significantly negative correlation between serum leptin levels and knee cartilage volume in olderadults, and serum levels of leptin were consistently associated with reduced cartilage thickness cross sectionally and longitudi-nally. Griffin *et al.* reported that the incidence of knee OA was not significantly different between leptin-deficient or leptin receptor deficient female mice and wild-type mice although body adipose tissue was increased by 10-fold in leptin-deficient or leptin receptor deficient mice. In contrast, a recent study showed that a fat-related increase in blood levels of IL-6 was associated with decreased physical function and frailty. Higher level of IL-6 was associated with an increased risk of knee OA progression, and serum levels of IL-6 and TNF-a were associated with joint space narrowing and cartilage loss in older adults65. A number of studies reported that IPFP secreted more IL- 6, IL-8, and prostaglandin E2 than ScAT in patients with knee OA (Liangjing, 2019).

Fibrosis, which is considered as a major pathological change in the local adipose tissue and a potential mechanism underlying knee OA, has been recently proposed. Histological analyses indicated that the extent of fibrosis in local adipose tissue was higher than that in SAT. Adipose fibrosis can be observed as hypo intense signals within the IPFP on MRI. Hypo intense signals in the IPFP assessed by MRI were significantly associated with higher risks of symptoms and structural damages of knee OA in a cohort study. A Recent study investigated fibrotic and inflammatory gene expression in IPFP and ScAT in mice that developed early OA after feeding with a high-fat diet for 20 weeks. The results showed an increase in fibrosis but no change in inflammatory markers in IPFP, compared to other two types of adipose tissues, suggesting that pro fibrotic IPFP remodeling without classic inflammation precedes knee OA in mice with diet-induced obesity (Chang, 2018; Mohsen Emadedin, 2018).

Tissue engineering approaches for OA cartilage repair have focused on cell-based therapies involving multipotential mesenchymal stem cells (MSCs). In adipose tissue, 5% of nucleated adipocytes are considered to be adipose tissue-derived stem cells (ADSCs). ADSCs are attractive sources of MSCs for the treatment of OA and can be easily harvested from ScAT. ADSCs have an enormous capacity of proliferation and differentiation to chon-drocytes72. Intra-articular administration of ADSCs obtained from ScAT can improve OA cartilage lesions in

rabbits. Percutaneous injection into joints with the autologous ADSCs with platelet-rich plasma resulted in significant decrease in visual analogue scale pain score in 91 patients with chronic joint disease and structural damages of knee OA in a cohort study. A Recent study investigated fibrotic and inflammatory gene expression in IPFP and ScAT in mice that developed early OA after feeding with a high-fat diet for 20 weeks. The results showed an increase in fibrosis but no change in inflammatory markers in IPFP, compared to other two types of adipose tissues, suggesting that pro fibrotic IPFP remodeling without classic inflammation precedes knee OA in mice with diet- induced obesity (Chang, 2018; Mohsen Emadedin, 2018).

Stromal cells derived from the IPFP shared common surface markers with bone marrow stromal cells (BMSCs) and had capacity of differentiation towards chondrocytes, osteoblasts, or adipocytesin vitro. Later studies about the IPFP-derived stem cells have merged regarding their chondrogenesis capacities under different microenvironments and the purposes of these studies focused mainly on cartilage regeneration. Almeida *et al.* reported that freshCD44þ IPFP stromal cells were capable of producing a tissue in vivo that stained strongly for sulfated glycosaminoglycan and type IIcollagen, suggesting that IPFP stromal cells may promote chon-drogenesis. Ye *et al.* also demonstrated the combination of TGF-b3and bone morphogenetic protein-6 strongly promoted chondrogenesis from IPFP adipose stem cells in a 3D chitosan scaffold. To evaluate the difference of ADSCs isolated from ScAT and IPFP, Ding*et al.* investigated the chemotaxis of IPFP stromal cells to chondrocytes and reported that IPFP stromal cells had a higher capacity for chondrogenic differentiation than mesenchymal cells from body adipose tissue and bone marrow (Chang, 2018).

Mechanism of action bone marrow mesenchymal cell

The regenerative potential of BM-MSCs:

BM-MSCs exert their regenerative effects primarily through paracrine signaling mechanisms. They secrete a spectrum of soluble trophic factors, notably bone morphogenetic protein-2 (BMP2) and insulin-like growth factor-1 (IGF1). These factors play a pivotal role in enhancing cellular regeneration and triggering bone formation by stimulating the proliferation and differentiation of endogenous progenitor cells found in various tissues. Moreover, they mitigate inflammatory and immune reactions associated with Osteoarthritis (OA) supporting the generation of anti-inflammatory T-cells. To maintain consistency and validity in MSC classification, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) has established specific criteria.

Immunomodulatory effects:

BM-MSCs also exhibit immunomodulatory prowess by inhibiting the activation of T- and B-lymphocytes, a process associated with the production of inflammatory cytokines. This inhibition effectively curtails immune responses, thereby fostering immune tolerance. Additionally, BM-MSCs stimulate the production of anti-inflammatory interleukin-1 (IL-1).

Pre-clinical and clinical applications:

Promising preclinical studies involving BM-MSCs for cartilage repair in animal models have laid the groundwork for expanding clinical applications. These studies have demonstrated

encouraging results, prompting a surge in clinical trials. In clinical settings, BM-MSCs administered to patients with knee osteoarthritis (KOA) have exhibited adherence to damaged tissue surfaces. Moreover, they have demonstrated the ability to differentiate into chondrocytes, leading to anatomical restoration and significant improvements in pain relief and functional outcomes

Challenges and considerations:

However, it is worth noting that the applicability of BM-MSC treatments may vary across different OA grades. Several studies have raised questions regarding their effectiveness in all cases. Additionally, a wide array of outcome measures has been employed across research studies, with some relying on qualitative questionnaires such as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and Lequesne algofunctional indexes. This reliance on subjective measures may introduce unintended bias due to the potential influence of physicians on patient responses. To mitigate such biases, the digital administration of questionnaires, completed independently without external input, may represent a promising avenue for future research.

Mechanism of action on intracellular allogenic adipose tissue

Allogeneic ascs are multipotent cells capable of differentiating into various tissue types, including cartilage. When injected into the knee joint, they exert their therapeutic effects through multiple mechanisms (wang, 2017).

Chondrogenesis: ascs can differentiate into chondrocytes, the cells responsible for cartilage formation and maintenance. This property contributes to cartilage repair and regeneration.

Immunomodulation: ascs possess immunomodulatory properties, suppressing local inflammation within the joint. This helps reduce pain and halt the progression of koa.

Paracrine signaling: ascs secrete trophic factors, such as growth factors and cytokines, that promote tissue repair and modulate the local cellular environment.

Clinical outcomes: several clinical studies have investigated the efficacy of intra-articular asc injections in koa patients. While results may vary, a growing body of evidence suggests positive outcomes:

Pain reduction: many patients experience significant reductions in knee pain following asc treatment, which can improve their overall quality of life.

Functional improvement: asc therapy often leads to improved joint function, allowing patients to engage in daily activities with greater ease.

Cartilage regeneration: some studies report evidence of cartilage regeneration and increased joint space following asc treatment, indicating potential disease-modifying effects.

Safety: allogeneic ascs have demonstrated a favorable safety profile, with minimal adverse effects reported in most studies.

Future prospects:

The use of allogeneic ASCs for KOA treatment holds promise, but several avenues for further research and development are worth exploring:

Optimal dosage and timing: determining the most effective dosage and timing of asc injections is crucial for maximizing therapeutic benefits.

Patient selection: identifying which koa patients are most likely to benefit from asc therapy based on disease severity and other factors.

Combination therapies: investigating the potential synergistic effects of combining asc therapy with other treatments, such as physical therapy or growth factors.

Long-term follow-up: conducting long-term studies to assess the durability of asc-mediated improvements and their impact on disease progression.

Conclusion:

Intra-articular injection of allogeneic adipose-derived stem cells represents a promising avenue for treating knee osteoarthritis. These cells offer multifaceted mechanisms of action, including chondrogenesis and immunomodulation, which can provide pain relief, improve joint function, and potentially modify disease progression. While more research is needed to optimize protocols and evaluate long-term outcomes, the use of allogeneic ASCs holds significant potential in the quest to enhance the quality of life for KOA patients. BM-MSCs hold immense promise in stimulating regeneration and alleviating the debilitating effects of knee osteoarthritis. Their multifaceted regenerative and immunomodulatory properties make them a compelling subject of research and clinical investigation. However, further studies, utilizing standardized assessment tools, are necessary to better understand their potential across varying stages of osteoarthritis and to ensure the validity and consistency of research outcomes.

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PERSONALIZED CANCER THERAPY

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Abstract:

Conventional cancer treatments often have significant side effects and may not be effective against advanced or resistant forms of cancer. Achieving personalized cancer treatment necessitates a strong grasp of cancer genomics, proficiency in the analytical techniques employed in cancer research, comprehension of how targeted medications work, and the ability to process and interpret intricate datasets. The most significant hurdle often lies in acquiring the medications recommended based on a patient's tumor genetic makeup. This paper highlights the potential of personalized cancer therapy. It delves into the molecular basis of cancer, emphasizing the importance of understanding genetic mutations and biomarkers. The paper discusses various approaches to treating different types of cancer, including targeted therapies and immunotherapies. It also acknowledges the limitations of current personalized cancer therapy, such as the need for more comprehensive genomic profiling and the challenges of resistance. Lastly, the paper outlines future directions, including advancements in precision medicine and the integration of artificial intelligence in treatment decision-making.

Introduction:

Medical professionals in the field of oncology have acknowledged for a significant period that each patient with cancer is unique. Clinical presentation of the disease differs from one person to another, its progression takes a unique path for each individual, patients have varying needs, preferences, and tolerances, and the results of treatment can greatly vary, even when patients receive the same treatment regimen. The emergence of novel drugs, encompassing both antibodies and small molecules designed to target precise molecules and essential pathways vital for cancer's survival, has brought about a revolutionary shift in the treatment of certain cancers. This breakthrough has instilled optimism in numerous patients who were previously facing seemingly incurable cancers. As the molecular characteristics of each individual's tumor are likely distinct, the concept of personalized cancer care has evolved beyond merely grasping a person's preferences. It now involves comprehending the distinct susceptibilities of each person's tumour. To effectively implement personalized cancer care, a comprehensive understanding of cancer genomics is essential. This includes recognizing the strengths, weaknesses, and limitations of analytical methods used in cancer research, acknowledging the factors that introduce variability in molecular testing, such as variations in tissue handling and processing, possessing in-depth knowledge of how targeted drugs function, and having the capacity to gather, process, and comprehend complex datasets within a busy clinical oncology setting. As the availability of various tests like single gene and multiplex mutation analysis, large-scale next-

generation sequencing, and transcriptomic analysis continues to grow through hospital, cancer center, and commercial laboratories, oncologists are increasingly equipped to meet these demands.

Limitations of conventional cancer therapy

Conventional methods of cancer treatment have several limitations. These include the toxicities induced by chemotherapy, such as nephrotoxicity, hepatotoxicity, neurotoxicity, cardiotoxicity, and gastrointestinal toxicity. Additionally, conventional chemotherapy suffers from a lack of aqueous solubility, lack of selectivity, and multidrug resistance. Furthermore, a suppressed immune response can become a lethal side effect after repeated cycles of intensive treatment. Although targeted therapy drugs have had successes in selected types of cancer, they are not likely to replace cytotoxic agents in the foreseeable future. However, clinical trials have shown potent synergy between targeted molecules and traditional cytotoxic agents. Efforts have been made to enhance the treatment outcomes of advanced cancer conditions by combining traditional cancer treatments with different immunotherapeutic methods. These attempts have demonstrated promising synergistic effects. Nanotherapeutics is a rapidly progressing field aimed at overcoming the limitations of conventional chemotherapy, such as lack of selectivity, multidrug resistance, and poor aqueous solubility (Qiao *et al.*, 2016).

Molecular basis of personalized cancer therapy

Colourful molecular biology styles for cancer discovery and treatment have been developed, including targeting cancer stem cell pathways for cancer remedy, retroviral remedy, oncogene silencing, and differences in excrescence suppressor genes is the part of molecular biology in cancer treatment (Jain *et al.*, 2009).

Individualized (Personalized) cancer drug can have smaller side goods than other types of treatment. This is because it's designed to be more specific. An individualized treatment may affect healthy cells less and cells involved in cancer more. The genes set up to be shifted in cancer, both oncogenes andexcrescence suppressor genes, law for factors of the pathways that regulate the social and proliferation of cells in the body — in particular, the mechanisms by which signals from an adjacent cell can impel it to divide, separate, or die (Ann Hoeben *et al.*, 2021).

The molecular approaches used are new antineoplastic agents, ultramodern immunotherapy, and the remedial form of oncogenic inheritable lesions. Utmost antineoplastic agents interact with DNA in a manner that inhibits the replication of the excrescence cell genome is the molecular approach to cancer treatment. There are molecular emblems of cancer which constitute an organizing principle for attributing the complications of neoplastic complaint. They include sustaining proliferative signalling, escaping growth suppressors, defying cell death, enabling replicative eternity, converting angiogenesis, and cranking irruption and metastasis (Israel *et al.*, 2020).

Development of targeted curatives emphasize the need for a multi-omics approach, integrating DNA and RNA differences, allowing a better understanding of excrescence biology, intra-tumor diversity, and the development of vulnerable- defense mechanisms. This allows for

the identification of excrescence-specific biomarkers as well as the development of innovative treatment strategies optimizing response rates and circumventing remedy resistance and this evolving field of liquid necropsies, anon-invasive way to descry and diagnose cancers and the early development of treatment resistance.

T cell remedy (CAR T cell therapy), the process of reengineering a case's own vulnerable cells to attack cancer, is a true advance in immunotherapy. This remedy has formerly entered Food and Drug Administration blessing to treat blood cancers, and it holds enormous pledge for the treatment of solid excrescences. The two types of treatment most frequently used in perfection drug are targeted medicine remedy (medicines designed to attack a specific target on cancer cells) and immunotherapy (drugs used to help the body's vulnerable system attack the cancer).

Excrescences retain the capability to foray girding towel considerably; so, restorative resection may come insolvable. Some of the general treatments for gliomas are a combination of surgery, radiation remedy, and systemic chemotherapy, there's significant room for enhancement and invention in managing the treatment (Feins *et al.*, 2019).

An operation of nanotechnology which holds great pledge for revolutionizing medical treatments, faster opinion, medicine delivery, towel rejuvenescence, and imaging is Nano drug. Nanotechnology helps deliver the medicine to the targeted towel across the blood- brain hedge (BBB), control releasing the medicine, and avoid declination processes. The other performance of this system is toxin reduction of supplemental organs and biodegradability which uses nucleic acids as drugs are called gene therapy.

Several implicit targets for new medicines have been linked using high- outturn technologies, and several composites have lately been approved or are under disquisition. One of the most current molecular differences in solid excrescences are PIK3CA mutations.

The PI3K/ AKT/ mTOR (The phosphatidylinositol 3- kinase (PI3K)/ AKT/ mammalian target of the rapamycin (mTOR)) pathway is an intracellular signaling pathway intertwined in cell proliferation (Dimitrios *et al.*, 2014).

This pathway can be actuated at several points, but PIK3CA mutations and PTEN (Phosphatase and tensin homolog) function loss are the most frequent sensible molecular differences. For this reason, several handbasket trials have been conducted or are still ongoing to assess the part of PIK3CA impediments in several solid excrescences.

In luminal bone cancer, prevalence of PIK3CA mutations is about 30 in primary excrescences and metastases. In a phase III trial enrolling PIK3CA mutant luminal bone cancer cases progressing to hormonal treatment, adding alpelisib, a specific p110α PIK3CA asset, to fulvestrant significantly bettered antitumor exertion and progression-free survival) over a combination of placebo plus fulvestrant, leading to its nonsupervisory blessing.

Several tools and technologies are being used to detect molecular alterations in cancer patients, including next generation sequencing (NGS), RNA sequencing, immunohistochemistry, fluorescence in situ hybridization microarray, biomarker testing, genetic testing, subtyping or tumor marker testing, genome sequencing or genomic profiling, molecular profiling, and somatic

testing. These tools have greatly improved our capacity to detect predictive and prognostic molecular alterations, such as gene mutations, amplifications, and fusions, which has opened the door to personalized treatment. Additionally, preclinical models such as patient-derived xenografts and organoids, as well as analyses of circulating tumor DNA, may also provide insights into potential codrivers and resistance mechanisms (Hoeben *et al.,* 2021).

Personalized cancer therapy in different types of cancer

1. Lung cancer: The treatment of lung cancer is changing as a result of personalised medication based on tumour characteristics. This review offers a summary of evolving tactics in various phases of clinical research as well as methods that have been clinically proven and can be used to tailor treatment for lung cancer patients. The treatment of lung cancer, which is primarily diagnosed as adenocarcinoma, has significantly advanced over the past ten years, with developments ranging from the introduction of histology-based therapies to the identification of targetable mutations. Without diagnostic molecular pathology's simultaneous technological development, these recent findings would not be feasible. A small amount of tissue can be used to evaluate many molecular changes in a single sample utilising next-generation sequencing (NGS) technology. The strategy that is most suitable for use in clinical practise is selective examination of certain cancer genes as opposed to whole-genome evaluation (Moreia, 2014).

Precision medicine, also referred to as targeted therapy or personalised therapy, is a form of lung cancer treatment that adjusts the course of action in accordance with the unique features of each patient's illness. This strategy accounts for the precise genetic alterations, molecular anomalies, and other elements that fuel the development of the tumour. Doctors can choose therapies that are more efficient and have fewer adverse effects than conventional, one-size- fits-all treatments by being aware of these special characteristics.

- Genetic testing: Genetic testing is carried out to find particular mutations or gene abnormalities in cancer cells. These alterations could be what fuels the cancer's expansion. The EGFR (Epidermal Growth Factor Receptor), ALK (Anaplastic Lymphoma Kinase), ROS1, and BRAF mutations are frequent in lung cancer.
- Targeted medicines: Once the genetic alterations have been located, targeted medicines can be administered that are designed to specifically target these mutations. These treatments aim to stop the altered proteins from acting in a way that promotes the spread of cancer. Compared to conventional chemotherapy, targeted medicines are frequently more effective and have fewer adverse effects.
- Immunotherapy: Immunotherapy is a different personalised treatment strategy for lung cancer. Immunotherapy aids in the body's immune system's ability to identify and combat cancer cells. Checkpoint inhibitors, a form of immunotherapy, stop the action of immune-suppressing proteins, enabling the immune system to attack cancer cells more effectively (Moreia, 2014).

2. Breast cancer: Breast cancer is the most common cancer among women in Every eighth to tenth woman in industrialised nations is at danger of acquiring breast cancer, making it the most prevalent type of cancer in this population. It continues to be the leading cause of deathfrom

cancer for females between the ages of 35 and 55 around the world. In many industrialised nations, including the USA and England, the mortality rate for breast cancer has been declining in recent years. Two major theories have been advanced for this decrease in breast cancer mortality: advancements in adjuvant systemic therapy and effective early detection brought about by broad screening initiatives. Up to 70% of patients with early-stage breast cancer who do not have distant metastases appear to be cured. wo major advances in breast cancer treatment have recently changed therapy concepts dramatically (De Abreu and Schwartz, 2014).

Breast cancer patients who receive personalised therapy have their treatment plans customised to their tumour and overall health. This strategy seeks to reduce side effects while maximising treatment efficacy. The following are some essential components of breast cancer personalised treatment.

- Tumour molecular profiling: To pinpoint certain genetic alterations, gene expression patterns, and protein markers, tumours are molecularly examined. The best treatment options can be chosen using this information (De Abreu and Schwartz, 2014).
- Chemotherapy: Chemotherapy remains a standard treatment option, but the choice of drugs and dosage can be tailored based on the tumor's characteristics and the patient's health status (Onkologie, 2012).

3. Brain tumor: The ability of prescribed medication to cross the blood-brain barrier (BBB), tumor-specific therapeutic administration, movement within the brain interstitium, and resistance of tumour cells to medicines are only a few of the characteristics that have been studied as factors that restrict brain cancer treatment efficacy. Recent advances in the field of nano-biotechnology have led to the development of multifunctional nano-theranostics, which have shown promise as an efficient treatment for brain cancer with the lowest potential risk of side effects. This study suggests a thorough, thoughtful, and critical discussion centred on effective nano-enabled platforms, including nanocarriers for drug delivery across the BBB and nano-assisted therapies, while taking difficulties and state-of-the-art achievements into account (Uzilov *et al.*, 2016).

Glioma, a type of brain cancer, can be treated with personalised therapies that adjust a patient's course of treatment to the unique features of their tumour. This method creates a tailored and efficient treatment plan by taking into consideration variables like the tumor's molecular profile, genetic make-up, and patient's overall health. An overview of how brain cancer may benefit from personalised therapy is provided below:

- Radiation therapy: High-energy x-rays or other particles are used in radiation therapy to kill tumour cells. Radiation therapy is one method that doctors may employ to delay or stop the growth of a brain tumour. It is frequently administered following surgery and may also be combined with chemotherapy. A radiation oncologist is a medical professional who focuses on administering radiation therapy to remove tumours. External-beam radiation therapy, or radiation delivered from a machine outside the body, is the most used form of radiation therapy. Internal radiation therapy, also known as brachytherapy, is the term used when radiation therapy is administered via implants. A radiation therapy regimen, often

known as a schedule, typically has a predetermined number of sessions spread out over a predetermined amount of time (Uzilov *et al.,* 2016).

- Target therapy: Targeted treatment is an additional medicine used by doctors to treat cancer in addition to conventional chemotherapy. A treatment known as targeted therapy specifically targets the tumor's unique genes, proteins, or tissue environment that supports the growth and survival of the tumour. This form of therapy prevents the development and spread of tumour cells while minimising the harm to healthy cells

Clinical applications

Personalized cancer therapy has shown great potential in improving patient outcomes by tailoring treatment strategies to individual patients based on their unique genetic profiles. This approach considers the heterogeneity and genetic instability of cancer cells, which can vary not only between different types of cancer but also within a single tumor (Beckman *et al.,* 2012). One of the clinical applications of personalized cancer therapy is the identification of actionable mutations and the selection of targeted therapies. By analyzing the genetic profile of a patient's tumor, specific mutations or alterations can be identified that are driving the growth and progression of the cancer. This information can then be used to select targeted therapies that specifically inhibit the activity of these mutated genes or pathways (Beckman *et al.,* 2012). This approach has been successful in several types of cancer, such as breast cancer with HER2 amplification and lung cancer with EGFR mutations, where targeted therapies have significantly improved patient outcomes (Beckman *et al.,* 2012). Another application of personalized cancer therapy is the prediction of treatment response and theprevention of drug resistance. By analyzing the genetic profile of a tumor, it is possible to identify genetic markers or signatures that are associated with treatment response or resistance. This information can be used to predict the likelihood of a patient responding to a particular treatment and to guide treatment decisions (Beckman *et al.,* 2012). Additionally, monitoring the genetic changes in a tumor over time can help identify the emergence of drug- resistant clones and allow for the adjustment of treatment strategies to overcome resistance (Beckman *et al.,* 2012). Furthermore, personalized cancer therapy can also be used to guide the selection of combination therapies. By analyzing the genetic profile of a tumor, it is possible to identify multiple genetic alterations or pathways that are driving the cancer. This information can be used to design combination therapies that target multiple vulnerabilities in the tumor, increasing the likelihood of a successful treatment response (Beckman *et al.,* 2012). In conclusion, personalized cancer therapy has several clinical applications that can significantly improve patient outcomes. These include the identification of actionable mutations and the selection of targeted therapies, the prediction of treatment response and the prevention of drug resistance, and the guidance of combination therapy selection. By tailoring treatment strategies to the unique genetic profiles of individual patients, personalized cancer therapy has the potential to revolutionize cancer treatment and improve patient survival rates.

Challenges and limitations:

Personalized cancer therapy has emerged as a promising approach in the treatment of cancer. However, there are several limitations that need to be addressed in order to fully realize its potential. One of the major limitations of personalized cancer therapy is intratumor heterogeneity. Intratumor heterogeneity refers to the presence of genetically distinct subpopulations of cancer cells within a single tumor (McGranahan & Swanton, 2017). This heterogeneity can arise due to genetic mutations, epigenetic changes, and clonal evolution (Marusyk & Polyak, 2010). It has been shown that intratumor heterogeneity can lead to the development of drug resistance and the failure of targeted therapies (Yap *et al.,* 2012). In addition, intratumor heterogeneity can complicate the identification of actionable mutations and the selection of appropriate targeted therapies (Fawdar *et al.,* 2013). Therefore, strategies to overcome intratumor heterogeneity and improve the efficacy of personalized cancer therapy are needed. Another limitation of personalized cancer therapy is the lack of effective drugs against most genomic aberrations. While there have been significant advancements in the development of targeted therapies, many genomic alterations still lack effective targeted drugs (Meric-Bernstam *et al.,* 2013). This limits the applicability of personalized cancertherapy to a subset of patients with actionable mutations. Furthermore, the high cost of targeted therapies and the challenges associated with reimbursement and regulatory approval can also hinder the widespread implementation of personalized cancer therapy (Meric- Bernstam *et al.,* 2013). Tumor heterogeneity, both inter- and intra-tumor heterogeneity, is another challenge in personalized cancer therapy. Inter-tumor heterogeneity refers to the differences between tumors of the same type, while intra-tumor heterogeneity refers to the heterogeneity within a single tumor (Zhang & Chen, 2018). Tumor heterogeneity can affect the response to treatment and the development of drug resistance (Zhang & Chen, 2018). It can also complicate the selection of appropriate biomarkers and the design of clinical trials (Zhang & Chen, 2018). Therefore, strategies to address tumor heterogeneity and improve patient stratification are needed to optimize personalized cancer therapy. In conclusion, while personalized cancer therapy holds great promise, there are several limitations that need to be addressed. These include intratumor heterogeneity, the lack of effective drugs against most genomic aberrations, and tumor heterogeneity. Overcoming these limitations will require advancements in our understanding of tumor biology, the development of novel targeted therapies, and the implementation of innovative clinical trial designs. By addressing these limitations, personalized cancer therapy has the potential to significantly improve patient outcomes.

Future directions:

The future of personalized cancer therapy holds several promising aspects:

1. Precision medicine advancements: As our understanding of cancer genomics deepens, personalized therapy will become even more precise. Therapies will be tailored based on an individual's unique genetic makeup, enabling targeted treatments that match the specific molecular characteristics of a patient's tumor.
2. Immunotherapy evolution: Immunotherapy will continue to advance, with improved

techniques for enhancing the immune system's ability to recognize and target cancer cells. Combination therapies that merge immunotherapy with other treatments will likely become more common, leading to enhanced responses.

3. Liquid biopsies and early detection: Liquid biopsies, which involve analyzing DNA, RNA, and proteins circulating in the bloodstream, offer a minimally invasive way to monitortumor progression and treatment response. These tests might also aid in early cancer detection.
4. Multi-omic integration: Integrating data from multiple omics sources (genomics, transcriptomics, proteomics, etc.) will provide a comprehensive view of a patient's cancer, allowing for more accurate treatment decisions.
5. AI and data analytics: Artificial intelligence and machine learning will play an integral role in processing the vast amount of complex data generated in personalized cancer therapy. These technologies will assist in identifying patterns, predicting treatment responses, and optimizing treatment plans.
6. Targeting resistance mechanisms: Resistance to therapies remains a challenge. Future personalized approaches will focus on identifying and targeting the mechanisms that lead to treatment resistance, improving long-term outcomes.
7. Microbiome influence: The gut microbiome's role in cancer progression and treatment response is being recognized. Future therapies might involve manipulating the microbiome to enhance treatment effectiveness.
8. Patient empowerment and education: With the availability of more information, patients will become active participants in their treatment decisions. Education and empowerment will be essential for informed choices.
9. Collaboration and data sharing: Collaboration among researchers, clinicians, and institutions will be crucial for advancing personalized cancer therapy. Open data sharing will facilitate the development of more effective treatments.
10. Evolving regulatory frameworks: Regulatory agencies will need to adapt to the dynamic landscape of personalized therapies, ensuring that new treatments are rigorously evaluated for safety and efficacy.
11. Accessible and affordable therapies: As personalized therapies become more prevalent, efforts to make them accessible and affordable will be essential to ensure that patients from diverse backgrounds can benefit
12. Rare and Pediatric Cancers: Personalized therapy approaches will be extended to rare and pediatric cancers, providing tailored treatments for patient groups that historically lacked options.

Conclusion:

In conclusion, personalized cancer therapy is a groundbreaking approach that takes into account a patient's individual characteristics to deliver more effective and precise treatments. By analyzing a patient's genetic makeup, doctors can identify specific mutations and biomarkers that drive cancer growth, allowing for targeted therapies and immunotherapies. This approach has the

potential to revolutionize cancer treatment, improving patient outcomes and quality of life. As research and technology continue to advance, we can look forward to even more advancements in personalized cancer therapy. The future of personalized cancer therapy holds the promise of increasingly targeted, effective, and individualized treatments that harness the power of genomics, immunology, technology, and collaboration to improve outcomes for cancer patients.

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STEM CELL THERAPY FOR DIABETES MELLITUS

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Abstract:

Diabetes mellitus is the most common chronic metabolic disorder. Type 1 diabetes mellitus is insulin dependent due to which it is an auto-immune disorder. Type 2 diabetes mellitus occurs when there is insulin resistance. It is characterized by pancreatic beta cell loss thereby leading to long term complications. Stem cell therapy is safe and has potential for curing patients with diabetes mellitus. The therapeutic outcome was achieved by mesenchymal stem cells along with umbilical cord derived stem cells. Stem cell therapy has been applied in clinical trials and animal models which shows effects on reducing insulin dosage and improving beta cell function and glycemic control without increasing risk of complications. Stem cell based research and therapies are the future for treating diabetes mellitus.

Keywords: Type 1 diabetes mellitus, Type 2 diabetes mellitus, Stem cell therapy, umbilical cord stem cells, mesenchymal stem cells

Abbreviations: Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), Mesenchymal stem cell (MSC), Umbilical cord (UC), Stem cell (SC), insulin producing

Introduction:

Diabetes mellitus and obesity are one of the fastest growing health challenges of the twenty-first century. There is a strong connection between diabetes mellitus and obesity sharing risk factors like unhealthy diet and lack of physical activity. There is a significant percentage of individuals with type 2 diabetes mellitus who are also obese this highlights the role of excess body weight in increasing the risk of developing diabetes mellitus. According to a report from the International Diabetes Federation, diabetes patients are expected to increase to 538 million by the year 2030 and the majority of people with diabetes are found in low and middle-income countries. This poses significant challenges in terms of health care resources and access to proper treatment. Diabetes mellitus is classified into four categories: Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), Gestational diabetes mellitus (GDM) and monogenic diabetes mellitus. Diabetes mellitus type 1 and type 2 are chronic metabolic disorders characterized by hyperglycemia that occurs due to elevated blood glucose level. Persistent hyperglycemia results in diabetic complication such as micro vascular disease (diabetic nephropathy, neuropathy, retinopathy and foot ulcer) and macro vascular diseases (cardiovascular diseases and peripheral vascular diseases) (Fig. 1) [1].

Pathophysiology of diabetes

Type 1 Diabetes Mellitus (T1DM)

It is an auto-immune condition in which the body's immune system mistakenly attacks and destroys pancreatic beta cells leading to a lack of insulin production and hyperglycaemia.

This leads individuals to rely on external insulin administration for proper glucose metabolism. Thus type 1 diabetes mellitus is often referred to as "Insulin dependent diabetes mellitus". Type 1 diabetes mellitus (T1DM) mostly occurs in adolescents and it equally affects both females and males. It can lead to a decreased life expectancy of about 13 years. Additionally, a small percentage of adults initially diagnosed with type 2 diabetes may actually have type 1 diabetes mellitus or latent autoimmune diabetes of adults (LADA). T1DM has increased prevalence of the condition among relatives indicates a genetic risk. A major genetic locus contributes significant risk for around 50-60% of genetic susceptibility. Environmental factors play an important role in the development of type 1 diabetes by interacting with genetic factors [1]. These environmental influences can trigger autoimmune responses that lead to destruction of pancreatic beta cells [2]. Some important environmental associations with type 1 diabetes are viral infections as well as changes in the composition of intestinal microbiomes. The timing of exposure to certain foods such as gluten could impact autoimmune response. Additionally low serum levels of vitamin D have been associated with type 1 diabetes. Intensive therapy required for individuals with type 1 diabetes involves administering external insulin. The primary objective of intensive insulin therapy is to keep blood glucose level as close to normal while preventing hyperglycemia [1].

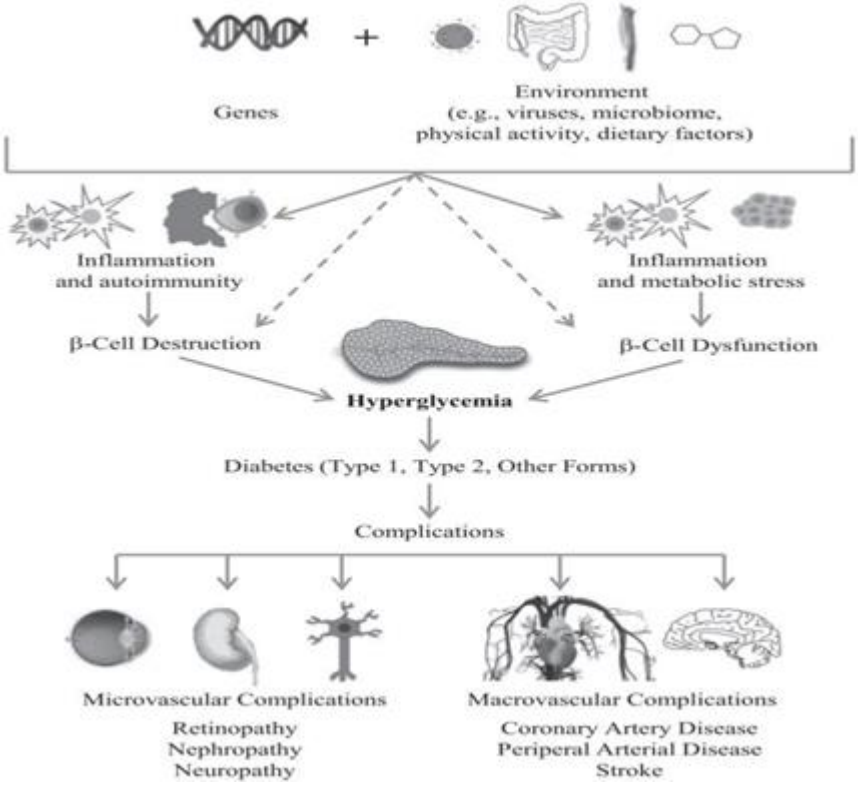

Fig. 1: Diabetes mellitus related health risks [1]

Type 2 Diabetes Mellitus (T2DM)

Insulin resistance plays a crucial role in the development of type 2 diabetes mellitus (T2DM). It occurs when the body's cells become less responsive to insulin leading to higher levels of glucose in the blood. This is called hyperglycaemia. Type 2 diabetes often develops gradually and is commonly associated with being overweight. The excess fat makes it difficult for the body to use insulin effectively. An estimation 90% of individuals living with diabetes

have type 2 diabetes mellitus. The prevalence of type 2 diabetes is generally higher for males than for females. The low socioeconomic status has increased risk of developing type 2 diabetes. Factors such as low educational level, income and occupation contribute to this risk. The heritability of type 2 diabetes has various factors such as gene – gene interactions (how different genes work together) and epigenetic (changes in gene expression caused by external factors). Obesity is indeed a significant risk factor for type 2 diabetes mellitus influenced by environmental factors. Insulin resistance often arises due to accumulation of fat in liver and muscle leading to decreased beta cell functions, inflammation within islets and eventually beta cell loss. Sleep deprivation and irregular sleep patterns can disrupt hormonal balance and affect insulin sensitivity, potentially contributing to development of diabetes. The agents to treat hyperglycemia in type 2 diabetes mellitus are metformin, DPP – 4 inhibitors, GLP – 1 receptor. Patients with type diabetes mellitus may require insulin therapy when beta cells are no longer able to function effectively [1,2].

Stem cells

Stem cells play a crucial role in regenerative medicine due to their unique abilities for self – renewal and differentiation into various cell types [4]. Stem cell therapies aim to replace non functional or deceased cells and tissues with functional ones, alter tissues microenvironment via anti – inflammatory and immunosuppressive effects and trigger self repair mechanisms.

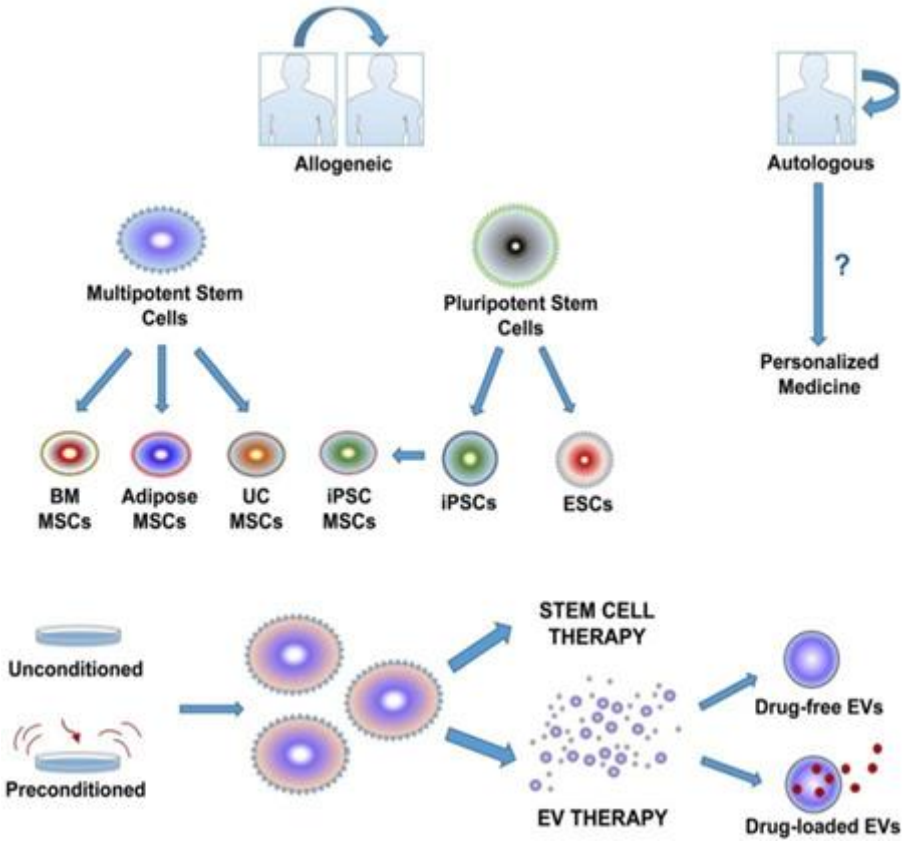

Fig. 2: Stem cells [3]

These attributes have led to extensive investigation in both experimental research and clinical trial for condition like cancer, cardiovascular diseases and issue related to liver, kidney, lung, orthopedic and skin diseases. Stem cell therapy avoid serious drawback such as limited

supply of organ donors and immune rejection [3,5]. Stem cells are classified based on their tissue of origin and differentiation potential. Embryonic Stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are pluripotent, capable of differentiating into a wide range of cell type including those derived from the germ layers (mesoderm, ectoderm and endoderm). Hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are multipotent, able to differentiate into various cell types. Multipotent stem cells are found in various tissues such as adipose tissue, umbilical cord blood and bone marrow (Fig-2) [3].

Sources of stem cell

There are various types of stem cells, including embryonic stem cells (ESCs), adult stem cell, induced pluripotent stem cells (iPSCs, mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). Each has distinct characteristics and potential application in medical research and treatment.

Embryonic Stem Cell (ESCs)

They are indeed pluripotent which means they have the potential to differentiate into cells from all three embryonic germ layer ectoderm, mesoderm and endoderm. These characteristics give them the ability to develop into a wide variety of cell types found in the human body. Embryonic stem cells have the potential to differentiate into insulin secreting cells which are important for treating diabetes. hESCs are short for human embryonic Stem cells. They can be guided to differentiate into renal lineage by exposing them to specific growth factors that mimic the developmental signals required for kidney cell formation. This process allows researchers to generate functional kidney cells for various applications such as drug testing [3].

Induced Pluripotent Stem Cells (iPSCs)

They are indeed adult cells that have been reprogrammed to exhibit characteristics similar to embryonic Stem cells. This reprogramming involves introducing specific transcription factors and protein to guide the adult cell towards a pluripotent state. Human induced pluripotent stem cells have demonstrated their effectiveness as a cell source for generating beta-like cells that respond to glucose levels. This has promising implications for potential diabetes treatment and research. The utilization of induced pluripotent stem cells for therapeutic purposes does indeed come with several challenges including potential autoimmune reactions, the risk associated with introducing foreign tissue into the body and the possibility of tumor formation from incompletely differentiated cells [3].

Mesenchymal Stem Cells (MSCs)

They are multipotent stem cells derived from the connective tissue or stomach surrounding the body's organs and other tissue. Mesenchymal stem cells have shown remarkable potential in regenerative medicine. Their ability to differentiate into various cell types make them valuable for generating tissues like skin, bone and cartilage for patients in need of reconstruction due to diseases or injuries. Additionally, ongoing research into mesenchymal stem cells therapeutic applications for autoimmune disease like multiple sclerosis holds promise for new treatment approaches [3,4].

Hematopoietic Stem Cells (HSCs)

They are precursors located in the bone marrow capable of maturing into various blood cell types like red blood cells and white blood cells as well as platelets. They possess self renewal ability, allowing them to divide and generate identical copies. Intermediate progenitor cells bridge the gap between hematopoietic stem cell and mature cell showcasing both multipotent and lineage committed traits either together or separately before completing maturation. The short life span of blood contributes to its high regenerative capacity while the bone marrow facilitates the movement of diverse cells to maintain blood cell homeostasis [3]. Both non – malignant conditions (like sickle cell disease) and malignant diseases (like leukemia) are addressed using hematopoietic stem cells. These cells replace or reconstruct the hematopoietic system of patients. Bone marrow or stem cell transplant are employed for treating individuals with both non – malignant and malignant diseases [5].

Adult stem cells

Adult stem cells possess the ability to continuously divide and self renew, allowing them to create diverse cell types from the same organ or even restore the entire original organ. This process of division and regeneration is the mechanism behind healing skin wounds and the ability of organs like the liver to recover from injury [3].

Experiment with Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells are also known as Mesenchymal stromal cells or medicinal signaling cells. Researchers have explored the potential of using mesenchymal stem cells (MSCs) derived from various adult human tissues to differentiate them into insulin producing cells as a potential treatment for diabetes mellitus [6]. Mesenchymal Stem Cells (MSCs) can be procured from various parts of the body including:

Umbilical cord and umbilical cord blood: mesenchymal stem cells from umbilical cord tissue and cord blood are known as Wharton's jelly – derived mesenchymal stem cells. These sources are rich in stem cells [7].

Bone marrow: it is a common source of mesenchymal stem cells. They can be isolated from the bone marrow and cultured for various applications including tissue regeneration.

Liver cell: hepatic cells which are found in the liver have been explored from their potential to differentiate into Mesenchymal stem cells (MSCs) – like cells and contribute to tissue repair.

Fibroblasts: Fibroblasts are connective tissue cells that can be reprogrammed into induced pluripotent stem cells (iPSCs) and then differentiated into various cell types including mesenchymal stem cells (MSCs).

Dental pulp and gingiva: the dental pulp and gingiva (gums) are emerging sources of mesenchymal stem cells that have been investigated for their potential in regenerative dentistry and other applications.

Placenta: Placenta tissue contains a population of stem cells. These cells have been investigated for their therapeutic potential

Bone marrow is indeed a rich source of Mesenchymal stem cells that have remarkable ability to self-replicate and differentiate into various cell types both in laboratory settings (in

vitro) and within the body (in vivo). Mesenchymal stem cells have remarkable ability to differentiate into multiple cell types known as multi – lineage differentiation is a factor that has led to their widespread popularity in research. They are easily cultivated and expanded, and even after extended periods of culture, they can maintain their pluripotentiality. Mesenchymal stem cells from bone marrow are often preferred for transplantation due to their immunological properties. They are considered immunological inert which means they are less likely to trigger immune responses. Additionally Mesenchymal stem cells possess immune suppressive qualities making them valuable where a perfectly matched donor is not available [4,6].

Mesenchymal Stem Cells (MSCs) transplantation

Mesenchymal stem cells have shown promise in cell therapy for diabetes mellitus. Mesenchymal stem cells share similarities with fibroblasts and are multipotent stromal cells. These cells can be sourced from different tissues such as bone marrow, adipose tissue and Umbilical cord blood. These cell sources are derived from healthy donors and tested negative for hepatitis B virus, hepatitis C virus and human immunodeficiency virus (HIV) [9]. Mesenchymal stem cells exhibit rapid differentiation along mesodermal lineages including the development of adipocytes, myoblasts, cardiac myocytes and even beta cell like cells. Bone marrow and Umbilical cord blood can be separated using density gradient centrifugation to collect the mononuclear cells and were washed with PBS (Phosphate buffered saline). The isolated mononuclear cells can be transferred to a 100 mm culture dish, where they can be stimulated to induce mesenchymal stem cells. The remaining adipose tissue or human umbilical cord tissue can be cut into small fragments and then placed in a 100 mm culture dish. This facilitates the collection of mesenchymal stem cells from tissue sources. The induced mesenchymal stem cells were preserved by storing them in liquid nitrogen. They were also cultured to prepare them for transplantation which would involve intravenous injection as a method of administration. The mesenchymal stem cells underwent flow cytometry analysis to evaluate their capability to express CD90, CD150, CD44 while showing an absence of CD34 and CD45 expression [8]. This assessment helps to confirm their suitability for therapeutic use. The potential of mesenchymal stem cells as a cell based therapy for treating immunologic disorder has been firmly established. Mesenchymal stem cells have the ability to modify the tissue microenvironment, supporting the survival and regeneration of beta cells. This effect can lead to an expansion in beta cell mass and ultimately facilitate the restoration of normal blood glucose level [8,9].

MSCs treatment for diabetes mellitus

Type 1 Diabetes Mellitus (T1DM)

Mesenchymal stem cells have shown promise in diabetes research. They are the most common donor cell for treating diabetes mellitus. Mesenchymal stem cell treatment could preserve beta cell function in new onset type 1 diabetes mellitus patients. Mesenchymal stem cells have been investigated for their potential to modulate the immune system and reduce the autoimmune attack on insulin producing beta cells in the pancreas. By doing so, they might slow down beta cell destruction. Patients undergoing treatment experienced significant improvement in their C-peptide and HbA1c levels. These positive outcomes suggest that the treatment has

contributed to better control of glucose level, resulting in reduced fluctuations and a decrease in the required dosage of exogenous insulin. These findings highlight in managing diabetes and improving overall glycaemic control. The absence of adverse events and the observed cases of insulin independence are positive indications of the potential benefits of mesenchymal stem cells treatment for type 1 diabetes mellitus [10,11].

Type 2 Diabetes Mellitus (T2DM)

Bone marrow–mononuclear cell (BM-MNC)

Bone marrow derived stem cell transplantation in type 2 diabetes mellitus can lead to a significant reduction in insulin requirement and HbA1c levels but bone marrow derived mononuclear cells therapy doesn't seem to have a significant effect on fasting C peptide level. The absence of serious chronic side effects and lingering effects suggest that bone marrow derived mononuclear cell therapy is both safe and effective, contributing to improvement of beta cell function in type 2 diabetes mellitus. Additionally the study indicates that higher infusion could potentially lead to greater effectiveness and correlation with reduced insulin requirement [12].

Mesenchymal Stem Cell (MSC)

Mesenchymal stem cells have demonstrated their potential in enhancing beta cell function and regulating the immune response. This indicates utilizing mesenchymal stem cells based approaches to potentially improve the management of diabetes. Throughout the follow up period notable improvements were observed including increase in C peptide level, a decrease in HbA1c level, blood glucose level, insulin usage and doses of oral antidiabetic medication. Furthermore there were positive changes in systemic immunological markers, which include reduction in T lymphocytes count and pro-inflammatory cytokines [13]. The safety and effectiveness of utilizing bone marrow mononuclear cells (BM-MNC) and bone marrow mesenchymal stem cell (BM-MSC) in type 2 diabetes mellitus. The patient who received a dose of 1×10^9/kg of bone marrow mononuclear cells (BM-MNC) and patient who received a dose of 1×10^6/kg of bone marrow mesenchymal stem cell (BM-MSC) experienced a noteworthy outcome during follow up: a significant reduction of over 50% in their exogenous insulin requirement. The study demonstrated not only a significant reduction in insulin requirement but also significant weight loss all without notable adverse side effects. These findings contribute additional support to the effectiveness and safety of mesenchymal stem cells and mononuclear cells transplantation for type 2 diabetes mellitus. The study also implies that combination of both MNCs and MSCs might yield improved outcomes and better control over glycaemia [14].

MSCs treatment for diabetic complication

Foot ulcer / Diabetic wound

Chronic skin ulcers are indeed a significant concern for individuals with diabetes often leading to serious complications due to the loss of dermal and epidermal tissue. Proper management of diabetes is essential to prevent development of foot ulcers. Diabetic wound healing deficiency can be attributed to various critical factors including reduced peripheral blood flow and impaired growth factor activity. Diabetes can disrupt the normal functioning of growth

factors that are essential for tissue repair and regeneration. Mesenchymal stem cells have shown potential in wound healing. They contribute to the improvement of dermal and epidermal junction leading to better overall wound healing. Additionally the use of mesenchymal stem cells has been associated with the formation of new skin structures like hair follicles which is a positive sign of tissue regeneration and recovery. The cytokines present in mesenchymal stem cell conditioned medium play a crucial role in wound repair and skin regeneration by reducing inflammation, encouraging the proliferation of skin stem cells and facilitating their differentiation into new keratinocytes which are essential for skin regeneration [15].

Conclusion:

Mesenchymal stem cells-based therapy has been considered a promising potential in regenerative medicine. They can differentiate into various cell types and modulate the immune response which makes them attractive for treating chronic disease like diabetes mellitus and its complications. The current clinical treatment for diabetes mellitus primarily involves insulin injection and hypoglycaemia agents to manage blood glucose levels. However these treatments often only provide temporary control and can lead to issues like weight gain. In various preclinical studies, Mesenchymal stem cells have demonstrated their effectiveness in reducing hyperglycemia by enhancing beta cell mass, improving insulin sensitivity and boosting glucose uptake and metabolism in peripheral tissues. Furthermore, Mesenchymal stem cells show potential in addressing diabetic complications through diverse mechanisms such as reducing inflammation and modulating the immune response. These include potential in aiding the healing of diabetic foot ulcers. The steady advancement in this field brings the possibility of a successful mesenchymal stem cell based therapy for diabetes mellitus and its complications a feasible objective in the near future.

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RESEARCH IN BIOMEDICAL, HEALTHCARE AND PHARMACEUTICAL SCIENCE

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Abstract:

Research is being done in all academic and developmental institutions but does not meet the expected level of scientific methodology. Biomedical research is a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge. The goal of personalized medicine is to tailor prevention strategies and treatments to each individual based on his/her genetic composition and health history. Research conducted to increase the base knowledge and understanding of the physical, chemical, and functional mechanisms of life processes and disease. It is also vital to record and assess experience in clinical practice in order to develop guidelines for best practices and to ensure high-quality patient care. Students will gain experimental as well as theoretical expertise in an area of concentration and are expected to develop the competencies needed for leadership in academia, industry and government agencies.

Keywords: Methodology, Genetic Composition, Systematic Investigation & Theoretical Expertise

Introduction:

Research is an integral part of learning, innovations, and developmental activities. Research is being done in all academic and developmental institutions but does not meet the expected level of scientific methodology. Moreover, the research carried out are not need based and lack in quality. The research should be directed towards major public health problems and aspects of health care.

There is need to create awareness and impart training in research to undergraduate, post graduate and health sciences students and professionals so as to motivate them for need based quality research in desired areas. Every treatment, intervention, medication, way of care, and aftercare in the medical field or health care system came from discoveries. This high quality of care we can experience today was not discovered overnight, but rather through years of effort by medical professionals who investigated the risk factors, causes, preventions, and treatments of diseases. This type of investigation is known as medical/health research.

Definition:

The general definition of research is, 'an investigation that is intentionally designed to help develop or contribute to knowledge'. When add a medical purpose to 'research', the general definition stays the same, but the goal becomes more specific. Ultimately, the goal shifts to a focus on increasing medical knowledge, improving patient care, developing new medicines or procedures, and enhancing the already existing medicines and procedures.

"A systematic, controlled empirical and critical investigation of hypothetical propositions about the presumed relations among the natural phenomenon."

- FN Kerlinger

"A fundamental state of mind involving continual re-examination of doctrines andaxioms upon which current thoughts and actions are based, it is, therefore, critical of existing practice."

- Theobald Smith

Clifford Woody states that research should comprise of "defining and redefining problems, formulating hypothesis orsuggesting solutions, collecting organizing and evaluating data, making deductions and research conclusions andcarefully testing the conclusionin brief."

- Redman and Mory

Characteristics of research in healthcare:

- It demands clear statement of the problem.
- It establishes the relationship between cause and effect.
- It helps in generation of principles and theories of prediction.
- It is based on observational, experimental and empirical evidence.
- It requires deep knowledge of the subject.
- It should be objective and logical.
- It should be carefully recorded and reported.
- It is characterized by patience and unhurried activity.
- Researcher should avoid personal feeling and preferences.

Biomedical science:

"Biomedical research" is defined as "a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge."

This is a broad definition that may include,

- Biomedical research, epidemiological studies
- Health services research
- Studies of behavioural, social, and economic factors that affect health.

Forms of health research:

1. **Clinical trial** - The patients volunteer to participate in studies to test the efficacy and safety of new medical interventions.
2. **Information based health research**- A great deal of research entails the analysis of data and biological samples that were initially collected for diagnostic, treatment, or billing

purposes, or that were collected as part of other research projects, and are now being used for new research purposes.

3. **Secondary use of data** - is a common research approach in fields such as epidemiology, health services research, and public health research, and includes analysis of patterns of occurrences, determinants, and natural history of disease; evaluation of health care interventions and services; drug safety surveillance; and some genetic and social studies.

Purposes of research in healthcare and biomedical science:

- Advances in information-based medical research could also facilitate the movement toward personalized medicine, which will make health research more meaningful to individuals.
- The goal of personalized medicine is to tailor prevention strategies and treatments to each individual based on his/her genetic composition and health history.
- Research conducted to increase the base knowledge and understanding of the physical, chemical, and functional mechanisms of life processes and disease.
- It is fundamental and not directed to solving any particular biomedical problem in humans or animals.
- This type of research often involves observing, describing, measuring, and experimental manipulation and provides the building blocks upon which the other types of research (applied and clinical) are based.
- A basic researcher seeks to add to the store of knowledge about how living things work.

Importance of health research:

- Like privacy, health research has high value to society.
- It can provide important information about disease trends and risk factors, outcomes of treatment or public health interventions, functional abilities, patterns of care, and health care costs and use.
- The different approaches to research provide complementary insights.
- Clinical trials can provide important information about the efficacy and adverse effects of medical interventions by controlling the variables that could impact the results of the study, but feedback from real-world clinical experience is also crucial for comparing and improving the use of drugs, vaccines, medical devices, and diagnostics.
- Therefore, tracking clinical experience with the drug is important for identifying relatively rare adverse effects and for determining the effectiveness in different populations or in various circumstances.
- It is also vital to record and assess experience in clinical practice in order to develop guidelines for best practices and to ensure high-quality patient care.
- Collectively, these forms of health research have led to significant discoveries, the development of new therapies, and a remarkable improvement in health care and public health.
- Economists have found that medical research can have an enormous impact on human health and longevity, and that the resulting increased productivity of the population contributes greatly to the national economy.

Oversight of health research:

- Historical development of federal protections of health information in research
- The development of international codes, federal legislation, and federal regulation of human subjects often occurred in response to past abuses in biomedical experiments.
- Most of the current principles and standards for conducting human subjects research were developed primarily to protect against the physical and mental harms that can result from these types of biomedical experiments.
- Therefore, they focus on the principles of autonomy and consent.

Common rule:

- The common rule governs most federally funded research conducted on human beings.
- It aims to ensure that the rights of human subjects are protected during the course of a research project.
- The common rule stresses the importance of individual autonomy and consent; requires independent review of research by an institutional review board (irb); and seeks to minimize physical and mental harm.
- The common rule also specifies a number of elements that must be provided when informed consent is sought.

Quality improvement and health research:

Quality improvement has been defined as "systematic, data-guided activities designed to bring about immediate, positive change in the delivery of health care in a particular setting".

Biomedical research:

The area of science devoted to the study of the processes of life, the prevention and treatment of disease, and the genetic and environmental factors related to disease and health.

Types of biomedical research:

Basic or "pure" research:

Research conducted to increase the base knowledge and understanding of the physical, chemical, and functional mechanisms of life processes and disease. It is fundamental and not directed to solving any particular biomedical problem in humans or animals.

This type of research often involves observing, describing, measuring, and experimental manipulation and provides the building blocks upon which the other types of research (applied and clinical) are based.

1. **Applied research:**

Research that is directed towards specific objectives such as the development of a new drug, therapy, or surgical procedure. It involves the application of existing knowledge, much of which is obtained through basic research, to a specific biomedical problem.

Applied research can be conducted with animals, nonanimal alternatives such as computer models or tissue cultures, or with humans.

2. **Clinical research:**

Using the knowledge gained in basic and applied research to conduct research (generally with humans) in treating disease or dysfunction in a new way. Research that takes place in a

hospital or clinical setting and is focused on treating specific human and animal diseases and other ailments. It builds upon the knowledge learned through applied and basic research.

Clinical research is conducted on human beings and takes shape in treatments and drugs that directly improve human healthcare.

3. **Biological models system:**

A system that can be observed instead of the original system, human or animal, that is of ultimate interest to the research. Researchers use models because they help to answer questions that could not be answered using the original system with the technology and methods that exist.

By using a model, researchers increase their ability to isolate and study certain features that would be too complex to study or impossible to isolate in the original system.

Types of models used in biomedical research:

- Whole living animals (human and non-human)
- Living systems composed of samples from the original animal (i.e., tissue culture)
- Non-living mechanical or molecular systems
- Mathematical models (i.e., computer simulations)

Biomedical & pharmaceutical research:

The Department of Biomedical and Pharmaceutical Sciences strengthens research through advances in Molecular Pharmacology, Neuroscience, Toxicology, Pharmaceutical Sciences, and Medicinal Chemistry.

A strong commitment to collaborative, multidisciplinary biomedical research has led to the development of Biomedical Research Excellence and leading research units.

Impact of biomedical and health research:

The impacts of research on the health of people in and around the world may not be measured by economic analyses, but historically these impacts have been among the most important benefits of research.

1. Public funding
2. Innovation system
3. Flow of knowledge
4. Funding for clinical trials and clinical research that informs clinical practice
5. Funding of other applications-oriented work, such as contracts to fund the development of technologies and to conduct consensus conferences.
6. Health policy
7. Biomedical technologies are the biggest source of long-run increases in health care costs.
8. Technology-driven cost increases may be unsustainable.

The drug industry relies mostly on medicine, biology and chemistry. The device industry relies on medicine and biology and, third, on materials science, which tends to be funded.

Drug and device innovation.

The Case of HIV drugs and vaccines

The role of the public sector in directly generating new drugs is much higher in HIV than in other arenas; nearly one third of drugs in this area rely on public sector research. Also, nearly

all commercially and therapeutically important vaccines over the last 25 years have come from the public sector, according to Stevens *et al.* (2011).

Device development

The scientific understanding holding consensus conferences to diffuse best practice and contracting with firms for device development and clinical trials. "The more applied side of the activities seems to be important".

Devices have important differences from drugs. And despite a good deal of discussion, there has not been much study of the effects of public sector research on health costs.

Types of study in medical research

Three main areas of medical research can be distinguished by study type:

- Basic (experimental),
- Clinical, and
- Epidemiological research.

Furthermore, clinical and epidemiological studies can be further subclassified as either interventional or noninterventional.

Gaps in monitoring science

The scientific community needs to do a better job of monitoring the state of science. Online science and social discussion groups and web-based communications from meetings and conferences could contain information useful to the policy community.

Further, basic and applied research tend to be artificially divided, but anything that is an innovation is going to go back and forth between the two categories of research.

Scope of biomedical science

The curriculum also covers medical ethics, clinical trials, and public health, providing students with a well-rounded education.

The growing healthcare sector in India presents a huge opportunity for biomedical engineers to develop products and solutions that can make healthcare delivery more efficient and effective.

Medical device innovation

Many people assume that the development of biomedical devices is similar to drugs, but in fact they have very different characteristics as they traverse the pathway from bench to bedside.

Drugs tend to be more discovery-based and derived from in-house activity. Devices are engineering-based. A specification is generated, along with an idea of how to realize that specification. Moreover, devices evolve over time. The first device is very different from subsequent generations, whereas a drug tends to be static for its lifetime.

A successful outcome for interventions using a medical device depends on rigorous manufacturing, which can be improved through federal research. Prototype products are often tested at these institutions, and even large companies may need to use academic centers for access to animals.

Criteria for biomedical research

1. Does the technology fit with a company's internal capabilities?
2. Is the fit with the customer good?
3. What is the market opportunity, including the number of customers, price, and the details of application?
4. Finally, what is the time to market, including the time needed to satisfy the regulatory process?

Making decisions in the pharmaceutical research:

The pharmaceutical industry and regulatory bodies need to evaluate drugs thoroughly and expeditiously as they go through years of clinical development before gaining approval for use in the treatment of a particular disease state.

It discovers and develops therapeutic products and technologies that are evaluated by regulatory agencies around the world to assess the efficacy and safety of a product for its intended use. In recent years, public confidence in the pharmaceutical industry and in the regulatory system has eroded. This may have the effect, if the regulatory process is lengthened, of delaying the introduction of innovative products or adding additional expense to the process and ultimately the final approved product or medicine.

The scope of research in pharmaceutical sciences ranges from identifying new drug targets and therapeutic agents to delivery and repurposing of drugs in clinical settings.

Research in pharmacology

Pharmacological research investigates the underlying mechanisms of interaction between drug molecules and the human body by developing advanced synthetic or biologically derived molecules that can treat or cure various pathophysiological conditions.

Issues in biomedical research:

- Lack of scientific training in research methodology.
- Insufficient interaction between the research institutions and other organizations/institutions.
- Duplication of research studies.
- There is no code of conduct for researchers.
- Inadequate secretarial assistance.
- Mismanagement off library functioning and publications.
- Lack of infrastructure.

Areas of research in biomedical, healthcare and pharmaceutical science:

1. Research in Clinical Practices
2. Research in Biomedical education
3. Research in administration
4. Research in Health systems and outcomes of care.

Research in clinical practices:

- ✓ Health promotion, maintenance, and disease prevention Patient safety and quality of health care.

- ✓ Promotion and risk reduction interventions of health of vulnerable, minority groups and marginalized community.
- ✓ Patient-centred care and care coordination
- ✓ Promotion of the health and well-being of older people
- ✓ Palliative and end-of-life care.
- ✓ Development of EBPs and translational research
- ✓ Care implication of genetic testing and therapeutics
- ✓ Nurses working environment care and community health care practices.
- ✓ Treatment compliance and adherence to treatment

Research in biomedical education

- ✓ Testing the effectivity and efficiency of the old teaching methods/techniques, and generating newer effective teaching tools and techniques.
- ✓ Curriculum taught and learning experiences.
- ✓ Enhancing the psychometric domain of learning in clinical practices.
- ✓ Extent of strictdiscipline required for the students to improve their learning and education.
- ✓ Promoting clinical and classroom learning among medical students.
- ✓ Refining and generating evaluation methods to judge the efficiency of the teaching-learning process.
- ✓ Identifying and managing problems of absenteeism and lack of motivation.
- ✓ Resolving any issue or phenomenon related to the teaching-learning process of the students.

Research in administration

- ✓ Assessing existing organizational structure, span of control, communication, staffing pattern, wages, benefits, performance evaluation practices, etc., and their effectiveness. In addition, developing new knowledge or refining the old knowledge regarding administrative phenomena.
- ✓ Developing and testing different administrative models to enhance swift administration, employees, and customer satisfaction.
- ✓ Recruitment, deployment, retention, and effective use of health care personnel in providing quality health care.
- ✓ Furthermore, research can be conducted on any phenomenon related to medical administrative issues.

Research in health systems and outcomes of care.

Research in health system and outcome of care is one of the important areas of research, where research scholars may identify the success of presently existing health care delivery systems and models in country and to identify the ways and means to develop affordable quality of health care to individual, families and communities of the country.

Healthcare research may not be completely separable from the biomedical research in this area, but it is integrated with health services research regarding issues of organization, delivery,

financing, quality, patient and provider behaviour, informatics, effectiveness, cost, and outcomes.

- Developing models of health care, which are affordable and accessible to people.
- Developing cost-effective model of health care for rural and deprived communities.
- Effective use of information and technology to provide health care services from tertiary care centres to remote and outreach areas.

Evaluate the effectiveness of existing policies and programmes for health care of people such as National Rural Health Mission (NRHM) and National Urban Health Mission (NUHM), etc.

Application of research in biomedical and pharmaceutical science:

The Department of Pharmaceutical and Biomedical Sciences (PBS) is an interdisciplinary program with basic science research spanning broad areas, including molecular pharmacology and pharmaceutics, drug discovery and delivery, protein engineering, and medicinal chemistry.

Areas of expertise that cross normal discipline limits include but are not limited to drug formulation, design and delivery, and injury prevention resulting from weapons of mass destruction and bioterrorism.

Students will gain experimental as well as theoretical expertise in an area of concentration and are expected to develop the competencies needed for leadership in academia, industry and government agencies.

Graduate students perform their dissertation research under the guidance of faculty engaged in diverse research spanning all major disciplines of pharmaceutical sciences. Most faculty members' research programs are highly interdisciplinary and collaborative with extensive overlap among the areas.

Research discoveries are central to achieving the goal of extending the quality of healthy lives. Research into causes of disease, methods for prevention, techniques for diagnosis, and new approaches to treatment has increased life expectancy, reduced infant mortality, limited the toll of infectious diseases, and improved outcomes for patients with heart disease, cancer, diabetes, and other chronic diseases.

Patient-oriented clinical research that tests new ideas makes rapid medical progress possible. Today, the rate of discovery is accelerating, and we are at the precipice of a remarkable period of investigative promise made possible by new knowledge about the genetic underpinnings of disease.

Forms of medical research

There are several forms of medical research being conducted today. Here are 3 common forms:

1. Basic or Laboratory-based research
2. Clinical Trials
3. Epidemiological Research

1. **Basic or laboratory-based research:** This is usually conducted in a laboratory where chemical interactions of biological materials are observed in a controlled environment. For most researchers, this is the first step toward developing methods or products that can be used in other forms of research studies.

2. **Clinical trials:** This is perhaps the most familiar form of healthcare research. Often, patients volunteer to participate in these studies to test the efficacy and safety of new medical interventions. Alternatively, medical interventions on participants may not be used, but only observation instead.
3. **Epidemiological research:** An increasingly large portion of health research is now information based. A great deal of research entails the analysis of data and biological samples that were initially collected for diagnostic, treatment, or billing purposes, or that were collected as part of other research projects, and are now being used for new research purposes. This secondary use of data is a common research approach in fields such as epidemiology, health services research, and public health research, and includes analysis of patterns of occurrences, determinants, and natural history of the disease; evaluation of health care interventions and services; drug safety surveillance; and some genetic and social studies.

Importance of research

- Medical research has led to many medical breakthroughs and developments.
- It would also strongly contribute to shaping the future of medicine

Medical research importance in disease diagnosis:

- Medical research has led to the development of diagnostic tools and technologies that allow for earlier and more accurate diagnoses of diseases.
- It's led to the development of an effective screening method known as mammography which has resulted in earlier detection and a 20% fall in mortality rates.
- A host of other effective screening methods have been developed as a result of medical research such as genetic testing, imaging techniques, and so on.

Importance of medical research in innovative treatments

- Medical research has led to the development of new treatments for a wide range of diseases, such as cancer, allergies, HIV/AIDS, heart disease, and so on.
- Research is essential to find out what treatments work best, and more specifically what treatments work best for what patient.
- It can provide important information about how effective a medical intervention is and its possible adverse effects. These interventions include drugs, vaccines, medical devices, and others.
- By being specific with participant requirements, medical professionals can study how certain groups of people react to certain treatments.
- Medical research would lead to newer developments in medicine such as personalized medicine and targeted therapies, that would ensure that each individual would have treatment options unique to them.
- Increasing research in this area is the only way to make this a reality in the future of medicine.

The role of medical research in disease prevention

- Medical research has contributed to the prevention of diseases such as polio, smallpox, and measles which caused the deaths of millions of people in the past.
- Recently, following the Covid-19 pandemic, medical research led to the development of vaccines that gradually slowed down the progress of the disease.

The importance of medical research in public health

- Medical research has contributed to our understanding of public health issues and how to address them.
- Research provides important information about disease trends and risk factors, outcomes of treatment or public health interventions, functional abilities, patterns of care, and health care costs and use.

Medical research's importance in improving the economy

- Medical research can have an enormous impact on the quality of healthcare which in turn affects human health and longevity.
- Healthy individuals tend to be more productive and that contributes greatly to the national economy.
- If the research enterprise is impeded, or if it is less robust, important societal interests are affected.
- Covid-19 vaccine development, for example, contributed to the lifting of the lockdown in many countries and allowed individuals to resume work.
- Compared to treatment, current research on disease prevention shows that preventive services are able to significantly reduce deaths and illnesses at reasonable costs.
- All of these findings have informed and influenced national budget planning and policy decisions.

Conclusion:

The simple fact is that clinical research improves our lives. It leads to significant discoveries, improves health care, and ensures that patients receive the best care possible. It is what makes the development of new medicines and treatments possible, without it we would not be able to move forward in the development of medicine. When we support, participate in, or conduct medical research, we are helping to continue to build the future of medicine.

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ALTERATION OF HEMOCYTE-MEDIATED CELL ASSEMBLAGE AND AGGREGATION DUE TO PYKNOSIS BY EXPOSURE OF ARSENIC AND MERCURY MAY AFFECT "ENCAPSULATION RESPONSE" IN EDIBLE MUD CRAB (*Scylla* sp.)

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Abstract:

The crustacean hemocytes play important roles in cell aggregation. The effect of arsenic and mercury on hemocytes was determined in edible mud crab (*Scylla* sp.) for 48 hrs and 72 hrs of span. Three morphotypes of haemocytes (hyaline, semigranular, and granular cells) were distinguished. As and Hg inhibited the degree of hemocyte aggregation and assemblage. Smaller poorly organized loose aggregates/assemblages were increased in treated group. The average number of pyknotic cells was increased in arsenic and mercury treatment. Hence, cell–cell aggregation is considered as an immunological response for host defence, the shift of cell assemblage may affect "encapsulation response".

Keywords: Hemocytes, Cell Assemblage, Aggregates, Pyknotic Cells

Introduction:

Heavy metals are typical environmental pollutants raising concerns over their potential effects on human health and bioaccumulation (Liu *et al.*, 2014). These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. Benthic crustaceans such as crabs act as biological indicators for monitoring the pollution status of heavy metals (Beltrame *et al.*, 2011). Crustaceans, like other classes of arthropods, have humoral and cellular defence mechanisms. Crustaceans display hemocytes (Smith, 1991), that are mainly subdivided into three cell types: hyaline, semigranular, and granular. In present study, impairment of cell assemblage/ aggregation response of hemocyte exposed to arsenic and mercury was suggestive of modulation of immune function.

Materials and Methods:

Edible mud crabs were purchased from a local fish market. The effect of arsenic and mercury on hemocytes was determined in edible mud crab (*Scylla* sp.) for 48 hrs and 72 hrs of span. In the laboratory, live crabs were maintained in glass aquaria. One group (n=6) was maintained in aquarium containing Arsenic Trioxide (ATO) in water at 5 mg/L and another group (n=6) was maintained in aquarium containing water using 50 ng/L $HgCl_2$ (Guria S *et al.*, 2023; Silveira de Melo Madson *et al.*, 2021). Control specimens were untreated by any toxic metals. Hemolymph of crabs was collected from the base of one of the second walking legs and hemolymph was smeared on glass slides, fixed by methanol and stained by Giemsa, Leishmans Eosin Methylene blue solution. The functional efficiencies of hemocytes were determined by

challenging the hemocytes with activated charcoal particles. The crabs were fed every day and the water was completely changed daily.

Fig. 1: Collection of hemolymph (indicated by arrow) of crabs from the base of legs

Results:

Microscopic observation:

Three morphotypes of haemocytes (hyaline, semigranular, and granular cells) were distinguished. Spindle shaped hyalinocytes involved in cell aggregation (Fig.2). Arsenic (As) and mercury (Hg) inhibited the degree of hemocyte aggregation and assemblage (Fig.3 and 4).

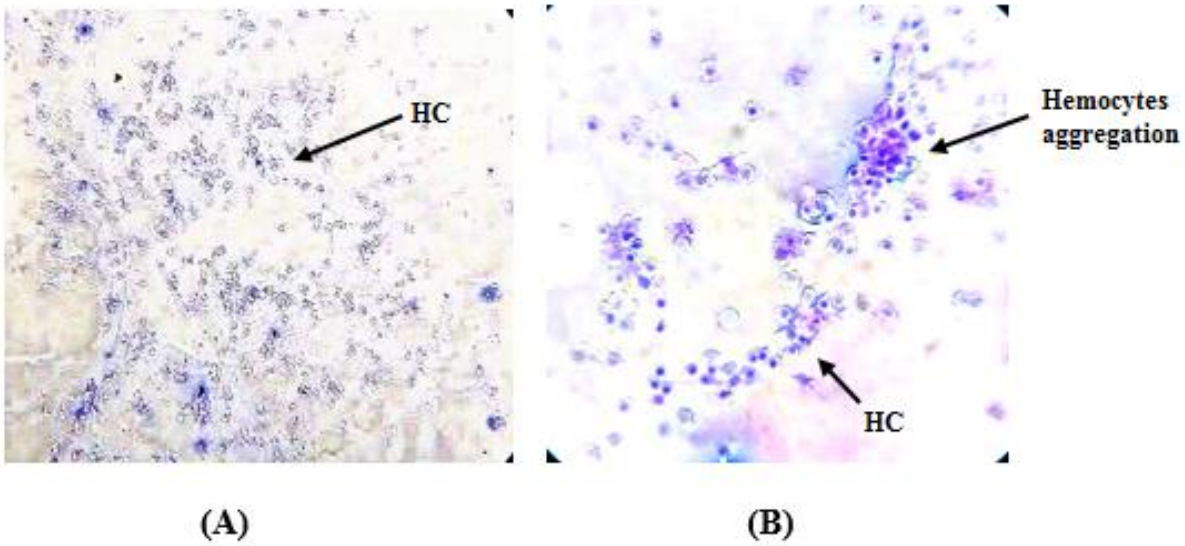

Fig. 2: Light micrograph of of hemocyte subpopulations. Spindle shaped hyalinocytes involved in cell aggregation (indicated by arrow).
(A) Giemsa stained cell assemblage/ aggregation in control hemocytes (x 100)
(B) Giemsa stained cell assemblage/ aggregation in control hemocytes (x 400)
HC=Hyaline Cells (Hyalinocytes)

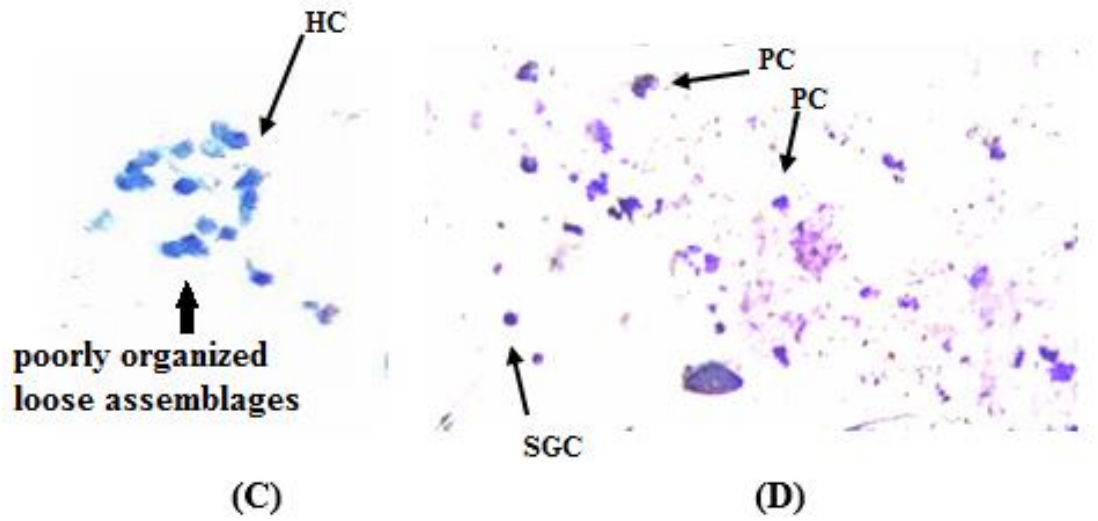

Fig. 3: As inhibited the degree of hemocyte aggregation (C, D).
(C) Leishmans Eosin Methylene blue solution stained smaller poorly organized loose aggregates/assemblages in As treated group in 48 hrs (x 400)
(D) Increased Giemsa stained pyknotic cells in As treated group in 72 hrs (x 400)
HC=Hyaline Cells, SGC= Small Granular Cells, PC=Pyknotic cells (indicated by arrow)

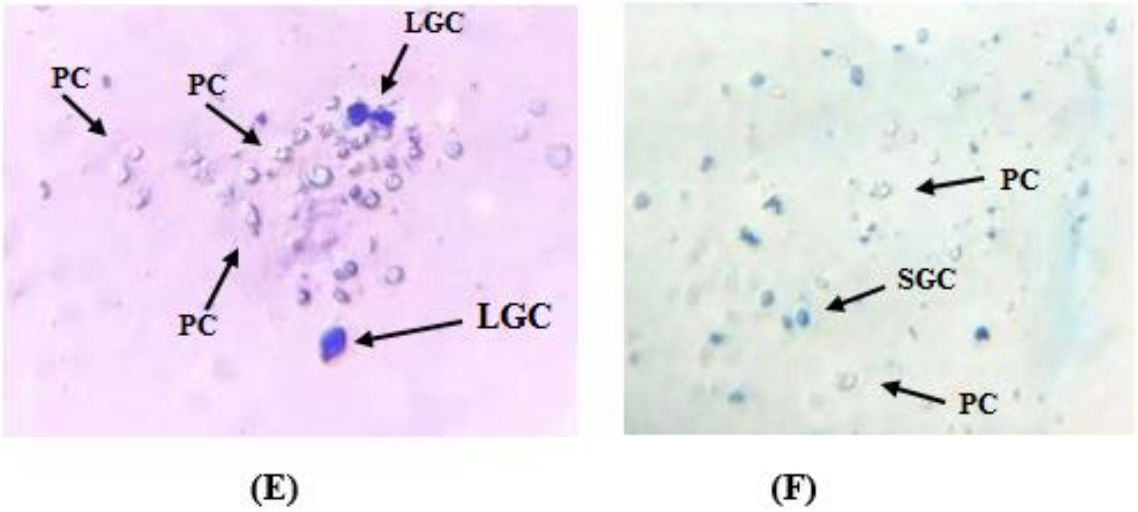

Fig. 4: Hg inhibited the degree of hemocyte aggregation (E, F)
(E) Giemsa stained smaller poorly organized loose aggregates/assemblages in Hg treated group in 48 hrs (x 400)
(F) Increased Leishmans Eosin Methylene blue solution stained pyknotic cells in Hg treated group in 72 hrs (x 400)
LGC= Large Granular Cells, SGC= Small Granular Cells, PC= Pyknotic cells (indicated by arrow)

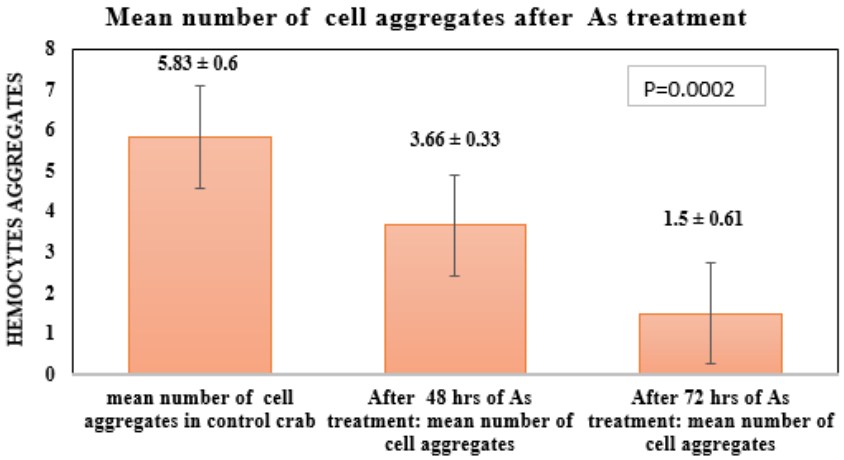

Fig. 5: Mean number of cells aggregates after As treatment noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

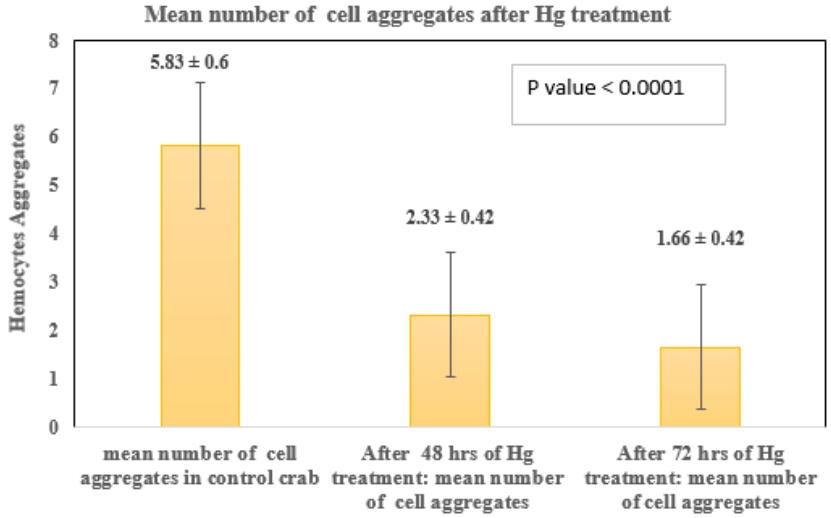

Fig. 6: Mean number of cell aggregates after Hg treatment noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

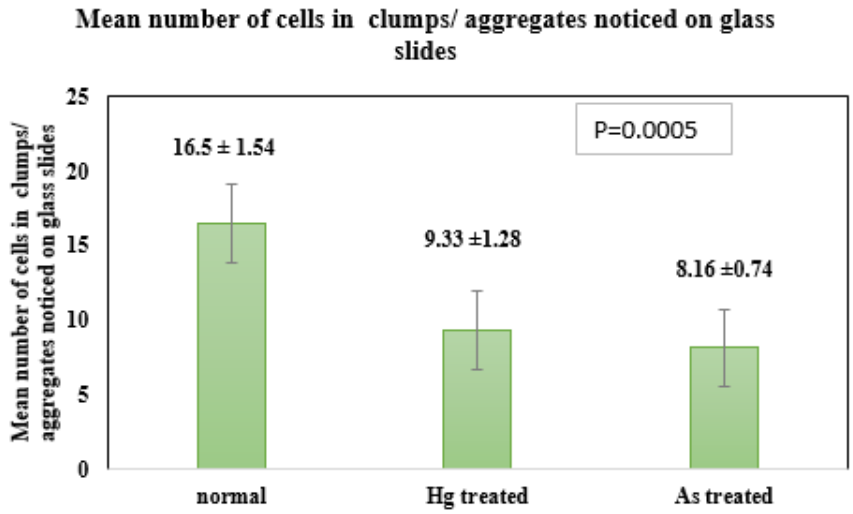

Fig. 7: Mean number of cells in aggregates after Hg and As treatment (after 72 hrs of exposure) noticed on glass slides. Values are expressed as Mean ± SEM. P-Value < 0.05 is considered to be statistically significant

Discussion:

Aggregation of haemocytes around invaded microorganisms is termed as "encapsulation response". Nodule and capsule formation have been most extensively studied in insects, but it occurs in many invertebrates, including crustaceans (Ratcliffe *et al.,* 1982). When the body cavity is invaded by particulate material such as bacteria, fungal spores or inert particles, in excess of those that can be removed by phagocytosis, the microorganisms become entrapped in several layers of hemocytes, that form a cell assemblage, or nodule (Battistella S *et al.*,1996).

Guria S *et al.,* 2016 reported the effect of arsenic on hemocytes of grasshopper and As inhibited the cellular aggregation in arthropod haemocytes. Guria S, 2020 showed hemocytes of *Pila* sp. yielded multiple morphological aberrations upon treatment with arsenic. Guria S, 2023 showed Lead (Pb) inhibited the degree of hemocyte aggregation in freshwater molluscs like *Bellamya bengalensis* which may affect "encapsulation response" and microaggregation.

In the present result, mean number of cell aggregates on glass slides was less after arsenic and mercury treatment (p value was 0.0002 in As treatment, p value was < 0.0001 in Hg treatment). Mean number of cells in clump/ aggregates was also decreased after treatment (p value was 0.0005). As a result, smaller poorly organized loose aggregates/assemblages were increased in treated group. The tendency of cell assemblage was decreased in 72 hrs of treated cells than 48 hrs of treated cells in both As and Hg exposure (Fig.3 and 4), as because the average number of pyknotic cells was increased in arsenic (As) and mercury (Hg) treatment.

Normally the nodule becomes heavily melanized due to host's phenoloxidase system. Encapsulation reactions, otherwise, are generally exhibited towards objects larger than 10 µm. The death of the encapsulated organism occurs by asphyxia or toxic action of quinones which are precursors of melanin (Battistella S *et al.*,1996). The shift of cell assemblage due to toxic exposure of heavy metal may affect "encapsulation response".

Acknowledgement:

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MURRAYA KOENIGII'S SECRET WEAPONS: TARGETING BREAST CANCER CELLS

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Abstract:

Treatment for breast cancer, the second most frequent cancer that mostly affects women, is extremely difficult. While effective, conventional methods like chemotherapy and radiation therapy frequently have negative side effects and are very expensive. Curry leaves, or *Murraya koenigii*, present a viable approach to overcoming these difficulties. This medicinal plant includes a wide variety of bioactive substances, such as terpenoids, alkaloids, and flavonoids. Particularly, isolated flavonoids from *M. koenigii* have demonstrated potential for reducing breast cancer cell proliferation. Furthermore, the high polyphenol content hydro-methanolic extract of curry leaves showed proteasome inhibitory action, causing cell cycle arrest and apoptosis in breast cancer cellsImportantly, the resistance of breast cancer stem cells to standard therapies frequently results in cancer recurrence. By inhibiting the Wnt/-catenin self-renewal pathway, koenimbin has demonstrated promise in the elimination of breast cancer stem cells. Additionally, *M. koenigii*'s aqueous extract has immunomodulatory and anti-inflammatory actions on breast cancer cells, which results in a decrease in the production of inflammatory cytokines and genes associated with them. In conclusion, *M. koenigii* has bioactive chemicals that may one day be used to treat breast cancer. To determine their effectiveness and safety, however, more research is needed.

Introduction:

Cancer is a condition brought on by the body's aberrant cells growing out of control. In the last few decades, factors such as changes in lifestyle, environmental pollution, urbanization, and changing food habits have led to an increase in the incidence of diseases, such as cancer (Nagappan *et al*., 2011; Noolu *et al*., 2013). Breast cancer is the second most lethal cancer after lung cancer (Noolu *et al*., 2013). Breast cancer is diagnosed in both men and women, but most commonly in women (Nagappan *et al*., 2011; Noolu *et al*., 2013). Genetic mutation, family history, estrogen levels, aging and sex are some risk factors linked to increase the possibility of breast cancer development (Wang *et al*., 2020). Despite their advantages, chemotherapeutic agents are relatively expensive and cause adverse effects, such as vomiting, nausea, hair loss, low blood count, diarrhea, and other severe adverse effects (Amna *et al*., 2019; Wang *et al*., 2020). Recurrence of breast cancer has been seen as chemotherapy and radiation therapy mostly unable to eradicate cancer stem cells (Ahmadipour *et al*., 2015; Wang *et al*., 2020). Cancer stem cells are types of cancer cells capable of forming an entire tumor again and are slow growing. Therefore, there is a need to develop safe, efficient, and beneficial alternative methods for the treatment and prevention of cancer recurrence. Traditionally, plants have been used as medicinal

sources for the treatment of many diseases. Different types of medicinal plants, herbs, and spices are promising sources of bioactive compounds with the potential to treat various diseases, including cancer. The use of natural products as anticancer agents will be a safe alternative as it shows less side effects compared to synthetic drugs (Amna *et al*., 2019). *M. koenigii* is one of plants having medicinal properties.

M. koenigii also referred to as "curry leaves," belongs to the Rutaceae family. *M. koenigii* is widely distributed throughout the tropical and subtropical parts of the planet. Asian cuisine frequently uses the condiment and spice *Murraya koenigii*. Because of their scent and medicinal qualities, fresh leaves from this plant are utilized in almost all curry and gravy recipes. It is a member of the Rutaceae family, is native to India, and is currently found over the majority of southern Asia (Amna *et al*., 2019). It is frequently employed to treat rheumatism, severe injuries, and snakebites. It has been established that this plant extract and a few isolated chemicals from it have anticancer potential, particularly in breast cancer. According to studies, this plant's leaves also possess a wide range of pharmacological advantages, including immunomodulatory, antibacterial, antifungal, anti-protozoal, antioxidant, and hypolipidemic activity. Despite the fact that this plant has produced a large number of phytochemicals, carbazole alkaloids have powerful pharmacological effects (Amna *et al*., 2019).

One of the best sources of carbazole alkaloids is the Murraya species. Several carbazole alkaloids from this plant, including mukonal, mahanine, and girinimbine, have demonstrated considerable anticancer efficacy, especially in breast cancer (Amna *et al*., 2019).

Taxonomy of plant

Plantae's Kingdom Tracheobionta Sub-Kingdom Magnoliophyta Division, Spermatophyta Superdivision Magnoliospida class Rosidae as a subclass, Sequence: Sapindales, the Rutaceae family Murraya J. Koenig ex. Genus.

Features of the plant

Curry leaves have 11–21 pinnate leaflets that range in size from 2-4 cm long to 1-2 cm wide. They taste weakly acidulous, bitter, and mildly pungent. (Ghasemzadeh *et al.,* 2014; Noolu *et al.,* 2013; Satyavarapu, Sinha and Mandal, 2020a). Curry leaves are frequently used as a flavoring ingredient in a variety of culinary dishes, including curries, chutneys, dals, and other foods (Yeap *et al.,* 2015).

Fig. 1. *M. koenigii* leaves (Noolu *et al.*, 2013)

Characteristic of *M. koenigii*

The *M. koenigii* plant, popularly known as curry leaves, is a little aromatic member of the Rutaceae family and the Murraya genus. Sanskrit refers to it as "Surabhinimba," Hindi calls it "Kari patta," and Tamil calls it "Karuveppilai or Karivepaku." (Kamalidehghan *et al.*, 2018; Satyavarapu, Sinha and Mandal, 2020a).

Individual compounds: The carbazole alkaloids *M. koenigii* contains include, among others, Mahanine, Mahanimbine, Girinimbine, Koenimbin, and Mukonal. There are flavonoids like quercetin, epicatechin, and myricetin. Additionally, phenolic acids have been found, including gallic acid and vanillic acid (Noolu *et al.*, 2013).

Bioactive substances: Numerous bioactive substances are present in the leaves, roots, and bark of *M. koenigii*, among other sections of the plant. Alkaloids, terpenoids, steroids, phenolics, flavonoids, tannins, and saponins are examples of secondary metabolites discovered in *M. koenigii*. The plant is full of vitamins and minerals like potassium, iron, calcium, phosphate, manganese, and magnesium in addition to dietary fibers, carbs, proteins, and lipids (Yeap *et al.*, 2015). (Satyavarapu, Sinha and Mandal, 2020b; Tanruean *et al.*, 2021; Wang *et al.*, 2020; Yeap *et al.*, 2015).

Medical qualities: Antioxidant, cytotoxic, antitumor, immunomodulatory, antiproliferative, antiangiogenic, antimetastatic, anti-inflammatory, antidiabetic, anticarcinogenic, antidysenteric, antihyperglycemic, hypoglycemic, and antimicrobial activities are just a few of the medical benefits of curry leaves. (Amna *et al.*, 2019; Ghasemzadeh *et al.*, 2014; Tanruean *et al.*, 2021; Wang *et al.*, 2020; Yeap *et al.*, 2015).

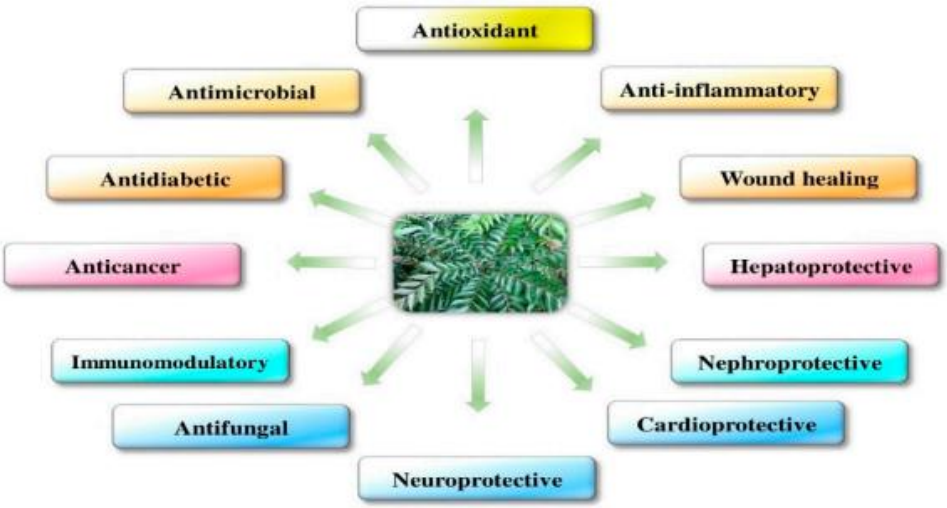


Fig. 2: *M. koenigii* (Pharmacological activities)
(Handral, H.K.; Pandith, A.; Shruthi, S.D.; 2012)

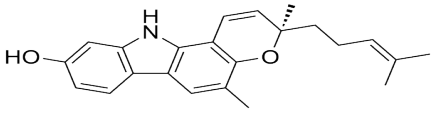
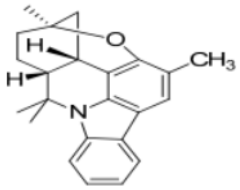
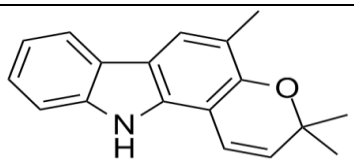
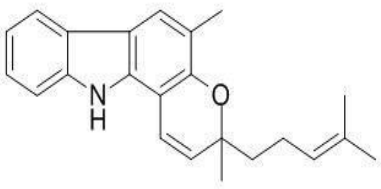
Phytochemistry of *M. koenigii* (Handral *et al.*, 2012)

- **Curry leave's phytochemical** makeup includes alkaloids, flavonoids, terpenoids, and polyphenols
- **Nutritional value:** The leaves are a good source of calcium, magnesium, salt, and several vitamins (A, B1, and B3)

- **The leaves' approximate composition is as follows**: Moisture: 63.2%; Protein: 8.8%; Carbohydrates: 39.4%; Fat: 6.15%; Sugars: 18.92%; Starch: 14.6%; Crude Fiber: 6.8%.
- **Chemical Components:** Carbazole alkaloids, essential oils, terpenoids, and flavonoids are among the main chemical components of *M. koenigii*

Phytochemicals	Values (mg/100g)
Alkaloids	1.90 ± 0.01
Saponins	2.50 ± 0.01
Flavonoids	7.43 ± 0.03
Tannins	0.86 ± 0.02
Phenols	4.25 ± 0.04
Glycosides	0.11 ± 0.01
Values are triplicate determinations and represent in Mean ± STD	

The major bioactive compounds of *M. koenigii* and its pharmacological activities

Sr no.	Bioactive Compound	Structure	Pharmacological activities
1	Mahanine		Cytotoxicity, anti-microbial, and anti-cancer
2	Girinimbine		**A**nti-tumor
3	Murrayafoline		Cytotoxicity and anti-tumor
4	Mahanimbine		Cytotoxicity, anti-oxidant, anti-microbial, anti-diabetic, and hyperlipidemic

Breast cancer

Breast cancer first appears in the breast tissue. Breast cells that mutate (change) and proliferate out of control result in a mass of tissue (tumor). Like other cancers, breast cancer can spread to the tissue around your breast. It might also spread to other parts of your body and cause the growth of new tumors. The medical word for this is metastasis The second most common

malignancy in women is breast cancer, following skin cancer. The majority of those affected are anticipated to be women over 50. Although uncommon, breast cancer can also strike men. Male breast cancer affects about 2,600 males annually in the US, accounting for fewer than 1% of total cases. Compared to cisgender men, transgender women are more likely to acquire breast cancer.

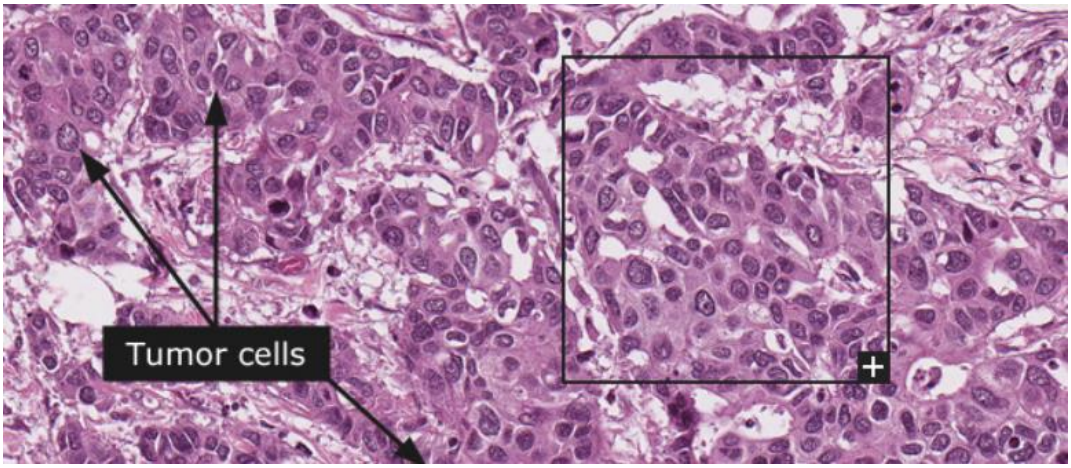


Fig. 3: Tissue section of breast cancer patient taken from (https://v16.proteinatlas.org/learn/dictionary/cancer/breast+cancer+3/detail+1)

Effects of bioactive compounds isolated from *M. koenigii* on breast cancer

Carbazole alkaloids, phenolics and flavonoids isolated from of *M. koenigii* possessed anticancer activity against various types of cancer. Effects of these bioactive compounds on breast cancer are as follows:

- **Growth inhibition by flavonoids:** Flavonoids are group of plants secondary metabolites which have wide range of biological properties most importantly antioxidant properties. It was found that extract of curry leaves containing flavonoid compounds such as myricetin, epicatechin, and quercetin had potency in the growth inhibition of MDA-MB-231 human breast cancer cell lines (Ghasemzadeh *et al.,* 2014). Flavonoids are capable of scavenging free superoxide radicals, providing anti anti-aging effect and reducing risk of cancer (Ghasemzadeh *et al.,* 2014).
- **Proteasome inhibitory activity of polyphenols:** 26S proteasome is a multienzymatic, multi-catalytic complex responsible for protein degradation which regulates cellular processes such as cell cycle, cell differentiation, signal transduction and apoptosis (Noolu *et al.,* 2013). In cancer cells proteasomes promote cell proliferation and protect cancer cells from apoptosis (Noolu *et al.,* 2013). Hydro-methanolic extract of curry leaves containing polyphenols, act as proteasome inhibitor; polyphenols decreases proteasome activity in breast cancer cells but not in normal fibroblast cells, arrest only cancer cells at S phase and cancer cells undergoes death via apoptosis (Noolu *et al.,* 2013).
- **Antiproliferative effect of Mukonal:** Mukonal is a carbazole alkaloid isolated from *M. koenigii* (Wang *et al.,* 2020). Apoptosis or programmed cell death is a neat, orderly process in which a sequence of events leads to death of irreparable cells. Mukonal induced apoptosis in breast cancer cells by enhancing expression of Bax (pro-apoptotic

proteins), cleavage of PARP and caspase-3 and retarding expression of Bcl-2 (anti-apoptotic) proteins in breast cancer cell lines (Wang *et al.*, 2020). Autophagy is a process in which cells degrade cellular components like some unwanted macromolecules or damage organelles. During autophagy autophagosomes are generated which contain cellular organelles to be degraded, later autophagosomes fuse with lysosomes to form autolysosomes in which components are degraded by lysosomal hydrolases. The formation of autophagosomes and enhanced expressions of Beclin-1, LC3B-I and LC3B-II proteins evident that mukonal induced autophagic cell death in breast cancer cells (Wang *et al.*, 2020). Thus, mukonal has shown antiproliferative effects on breast cancer cells by induction of apoptosis and autophagy.

- **Anti-inflammatory and immunomodulatory effect of aqueous extract of *M. koenigii*:** Yeap et al. (2015) studied anti-inflammatory and immunomodulatory effects of *M. koenigii* aqueous extract on breast cancer, by inoculated 4T1 breast cancer cell-challenged mice with aqueous extract of *M. koenigii*. Levels of NF-kB, iNOS, NO, IL-1β, IL-10 and IL-6 were evaluated in MK- treated and untreated mice (Yeap *et al.*, 2015). Nuclear factor-kappa B (NF-kB) is a family of transcriptional factors that play an important role in inflammation, cell proliferation and protects cells from apoptosis (Liu *et al.*, 2015). NF-kB can be activated by proinflammatory cytokine IL-1β and pleiotropic cytokine IL-6 (Yeap *et al.*, 2015). NF-kB is constitutively active in many tumors, leading to the upregulation of proteins encoding adhesion molecules, inflammatory cytokines growth factors, and anti-apoptotic proteins (Kamalidehghan *et al.*, 2018; Liu *et al.*, 2015). Overexpression of NF-kB promotes proliferation, anti-apoptosis, angiogenesis, invasion and metastasis in cancer cells (Liu *et al.*, 2015; Yeap *et al.*, 2015). Elevated level of serum IL-1β, IL-6 and IL-10 were observed in untreated 4T1 breast cancer cell-challenged mice (Yeap *et al.*, 2015). Inducible nitric oxide synthase (iNOS) catalyzes oxidative deamination of l-arginine to produce the pro-inflammatory mediator NO and raise in NO level is measured of the presence of inflammation in the serum and tumor which leads to tumor progression and metastasis (Yeap *et al.*, 2015). Intercellular adhesion molecules (iCAM) is a surface glycoprotein in immunoglobulin superfamily, expression of iCAM in tumor plays important role in tumor progression and metastasis as its over-expression promotes transendothelial migration of the cancer cell (Yeap *et al.*, 2015). c-MYC is a proto-oncogene, overexpression of leads to cell proliferation thus promoting tumorigenesis. Levels of NF-kB, iNOS, inflammatory cytokines (IL-1β, IL-10 and IL-6) and expression of iCAM and c-MYC was found to be effectively reduced in MK aqueous extract treated mice (Yeap *et al.*, 2015).
- **Induction of apoptosis by koenimbin:** Ahmadipour et al. (2015) studied in vitro effect of koenimbin a carbazole alkaloid derived from the plant *M. koenigii* against MCF7 cells and derived MCF7 stem cells/progenitors. It was found that after treating the cells with koenimbin, growth inhibition was seen in MCF7 cells and derived MCF7 stem cells/progenitors, while non- invasive MCF-10A cells were not affected significantly

(Ahmadipour *et al.,* 2015). Growth inhibition of cancer cells was due to induction of an intrinsic apoptotic pathway by koenimbin (Ahmadipour *et al.,* 2015). Koenimbin inhibits NF-κB translocation from the cytoplasm to the nucleus, downregulate Bcl2 (anti-apoptotic) and upregulate Bax (pro- apoptotic), leading to destruction of mitochondrial membrane permeabilization (MMP) causing release of mitochondrial cytochrome c to the cytosol which sequentially activates caspase-9 and caspase-7 and finally leads to apoptosis (Ahmadipour *et al.,* 2015). The Wnt/β-catenin signaling pathway plays a key role in promoting the self-renewal of breast CSCs (Ahmadipour *et al.,* 2015). Koenimbin also targets MCF7 CSCs by downregulating the Wnt/β-catenin self-renewal pathway (Ahmadipour *et al.,* 2015). Glycogen synthase kinase (GSK)3β increases the degradation of β-catenin through the ubiquitin–proteasome pathway via phosphorylation of three specific amino acids, i.e., Ser33, Ser3, and Thr41 (Ahmadipour *et al.,* 2015).

Uses for medicine

- **Flatulence and digestion**: Several plant parts, including the leaves, roots, and bark, can be used as tonics to promote healthy digestion and reduce flatulence. The leaves are bitter after infusion and are used to lower fever.
- **Renal pain management:** The root's juice is used to treat kidney (renal) discomfort.
- Curry leaves are used as anthelmintics, analgesics, piles treatments, body heat reducers, and thirst quenchers, among other medicinal purposes. They are also used to treat blood problems and leucoderma, as well as to lessen inflammation and irritation.
- **Treatment for dysentery and toxic bites:** Boiling green leaves in milk to make a paste is used to treat toxic bites and skin eruptions. Dysentery can also be treated by eating raw, uncooked greens.

Conclusion:

Curry leaves, or *M. koenigii*, have demonstrated tremendous promise for the treatment and prevention of breast cancer. Alkaloids, phenolics, flavonoids, and polyphenols, among other bioactive substances found in this medicinal plant, have shown a variety of anticancer activities against breast cancer cells. Apoptosis activation, growth inhibition, proteasome inhibition, autophagy induction, and manipulation of inflammatory and immunomodulatory pathways are a few of these characteristics.

Curry leaves contain flavonoids that have been demonstrated to stop the development of human breast cancer cell lines. As a result of the proteasome-inhibitory action of the polyphenols in the hydro-methanolic extract of curry leaves, breast cancer cells experience cell cycle arrest and apoptosis.

While koenimbin efficiently targets cancer stem cells by activating intrinsic apoptotic pathways and downregulating the Wnt/-catenin self-renewal pathway in breast cancer cells, carbazole alkaloids like mukonal also cause apoptosis and autophagy in breast cancer cells.

By lowering the levels of inflammatory cytokines and genes linked to inflammation in breast cancer cells, the aqueous extract of *M. koenigii* also demonstrates anti-inflammatory and immunomodulatory properties.

These results demonstrate the potential of *M. koenigii* as a useful natural resource for the development of safer and more efficient breast cancer alternative therapies. In order to create novel anticancer medications with fewer side effects and greater therapeutic advantages, more investigation is required to confirm the efficacy and safety of these bioactive molecules. The plant *M. koenigii* has the potential to enhance breast cancer treatment and lower the likelihood of cancer recurrence.

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STREAMLINING DRUG MANUFACTURING AND DRUG MANAGEMENT PROCESS

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Abstract:

This chapter provides an insightful exploration of the intricate journey that pharmaceuticals undertake from their inception in research laboratories to reaching the hands of consumers at the pharmacy counter. It delves into the multidimensional process of drug development, encompassing discovery, preclinical testing, clinical trials, regulatory approval, manufacturing, and the complex world of pharmaceutical marketing and distribution. The chapter unveils the collaborative efforts of scientists, researchers, regulatory agencies, manufacturers, and healthcare providers, shedding light on the challenges, innovations, and ethical considerations that shape this remarkable industry. Readers will gain a comprehensive understanding of the pharmaceutical ecosystem, its impact on healthcare, and its role in improving and sustaining human well-being.

Keywords: Pharmaceutical Innovation, Drug Development, Preclinical Testing, Clinical Trials, Regulatory Approval, Pharmaceutical Marketing, Patent Protection, Generic entry, NDA, FDA, Lifecycle management

Introduction:

The inception of a novel medicine often originates with scientists discovering a biological target, such as a receptor, enzyme, protein, or gene, associated with a malfunctioning biological process seen in diseases like Alzheimer's disease (AD). In this context, we focus on the exploration and advancement of entirely new medications, distinguished by their distinct modes of action from already approved drugs and tailored for clinical indications unaddressed by existing treatments. While medicines that represent incremental enhancements to current therapies hold value due to potential benefits in terms of potency, safety, tolerability, or convenience, they typically do not involve the manipulation of biological targets different from those affected by current medications. Comprehensive analyses across diverse therapeutic areas consistently indicate that the journey of developing a new medicine, spanning from target identification to marketing approval, spans a duration of over 12 years, often extending well beyond this timeframe. Upon approval, a molecule must adhere to stringent regulations governing its manufacturing, emphasizing purity and stability.

While manufacturing concerns are typically not within the purview of discovery biologists, the intricacies of producing a new medicine can prove intricate and costly, particularly in the case of biological products (NBEs). The intricacy of the manufacturing process often plays a pivotal role in determining the financial feasibility of investing in a specific biological target. Additionally, for a new medicine to gain acceptance within the medical

community, ensuring that physicians and patients can confidently administer it to the right individuals, in the correct dosages, and at the appropriate times is imperative. When new medicines are designed for use in a patient group already identified through widely adopted diagnostic practices, identifying the appropriate patients may pose minimal challenges. However, if the target patient population does not align with current diagnostic practices, proactive efforts are required to empower clinicians in identifying the right patients before the product's launch.(Richard C. Mohs, Nigel H. Greig, 2017)

The drug development process, spanning from pharmaceutical manufacturing to finalization, typically encompasses an approximate duration of 15 years. This intricate journey commences with the discovery and scientific research of pharmaceutical compounds, followed by meticulous preclinical investigations conducted within laboratory settings. These preclinical assessments yield essential data, subsequently compiled into an Investigational New Drug (IND) Application, which is then submitted to the U.S. Food and Drug Administration (FDA). Subsequent to the IND submission, clinical trials are meticulously executed. Clinical trials are typically divided into three phases to rigorously evaluate the safety and effectiveness of new treatments. Phase I involves a small group of healthy volunteers to assess safety, dosage, and side effects. Phase II expands the study to a larger group of patients to further evaluate safety and effectiveness. Phase III includes a much larger and diverse patient population to confirm the treatment's efficacy, monitor side effects, and compare it with existing treatments. These phases provide crucial data for regulatory approval and ensure that new therapies meet high standards of safety and efficacy before reaching the broader patient population. Following this, the drug undergoes scrutiny to assess its safety and efficacy, ultimately culminating in FDA approval, if deemed satisfactory. Post-approval, vigilant monitoring of the drug ensues over a specified timeframe, heralding the initiation of preparations for its market launch. Furthermore, securing a patent for the pharmaceutical is imperative within this multifaceted process.

The FDA plays a crucial role in overseeing the entire pharmaceutical lifecycle, ensuring rigorous standards for safety and quality. It evaluates IND Applications, monitors clinical trials, and assesses NDAs. The FDA also conducts inspections to enforce Good Manufacturing Practices, safeguarding medication quality. This vigilant oversight is essential for public health and pharmaceutical safety.

This chapter elucidates the sequential progression of these processes, commencing with the drug discovery phase and culminating in its market launch.

Discovery and research:

Drug discovery is the process of seeking and developing new medications or drugs to address various diseases and medical conditions. This involves the identification of molecules or compounds with the potential to cure, alleviate, or prevent illnesses. Scientists and researchers in this field look for substances that can target specific biological processes, such as blocking harmful proteins or enhancing beneficial ones. Once promising candidates are identified, they undergo extensive testing and research to ensure their safety and effectiveness for treating

patients. The ultimate aim of drug discovery is to enhance human health by creating new medicines that can combat diseases and enhance the quality of life for those in need.

The process of discovering new drugs encompasses multiple stages, each demanding a diverse set of skills and the utilization of advanced technological tools. It typically involves a combination of computational and experimental methods to validate potential drug targets and identify substances with therapeutic potential. As the process unfolds, initial experimental compounds undergo meticulous refinement to ensure they exhibit the necessary attributes of specificity, efficacy, and safety. This refinement is based on results obtained from initial in vitro tests and animal models. Once these compounds meet the required criteria, they are officially designated as drug candidates. At this juncture, the project's primary focus transitions from the earlier stages of drug discovery to the subsequent phase, which is drug development. This shift is pivotal, as it marks the preparation for human clinical trials. The goal of these trials is to rigorously evaluate the therapeutic agent's safety and efficacy in real human subjects under controlled conditions. If the therapeutic agent proves successful across all three phases of clinical trials, it advances to the final stages of the drug development process. This entails seeking regulatory approval, which involves extensive scrutiny by health authorities to ensure the medication meets safety and efficacy standards. Upon receiving regulatory approval, the drug is cleared for market entry, where it becomes available for patient use, contributing to the advancement of medical treatment options.

The process begins by focusing on a specific disease and identifying potential targets, often proteins that can be influenced by small compounds. These compounds are expected to either disrupt, alleviate, or at least slow down the disease's progression. Target identification involves various methods, such as cellular assays, genomic studies, and proteomic studies, among others. Following this, thousands, or even millions to billions when utilizing computer-aided drug design before in vitro assays, of small molecules undergo screening in various types of assays. A limited selection of promising molecules is then subject to further evaluation in animal models and alternative in vitro models that mimic human diseases. It's important to note that animal models can occasionally produce deceptive results; for example, a substance found to be toxic in animal models may not exhibit the same toxicity in humans, or vice versa. The objective of a preclinical drug discovery program is to present one or more clinical candidate compounds, each supported by substantial evidence of biological activity against a disease-relevant target, along with adequate safety and drug-like characteristics. This enables their progression into human testing. Typically, many discovery programs aim to generate multiple candidate molecules.(Singh Natesh, Vayer Philippe, Tanwar Shivalika, Poyet Jean-Luc, Tsaioun Katya, Villoutreix Bruno O., 2023)

Developers searching for treatments for conditions like Alzheimer's, cancer, and other complex diseases are eager to uncover fresh drug targets. Yet, they often view new scientific discoveries in the literature that suggest novel targets with caution. Given the significant time and financial commitment needed to investigate a new biological target, drug developers typically prioritize replicating reported findings before initiating screenings for new targets.

Unfortunately, even when attempting to reproduce results published in respected journals, a substantial portion, potentially up to 90%, cannot be replicated. (Richard C. Mohs, Nigel H. Greig, 2017)

After years of thorough research, a small number of compounds ideally exhibit the necessary safety and effectiveness to progress to clinical trials involving patients.

Pre-clinical testing:

Preclinical testing of a drug is a crucial phase in the drug development process. It involves a series of laboratory and animal studies to assess the safety, efficacy, and pharmacological properties of a potential drug candidate before it can advance to human clinical trials.(Andrew G Polson, 2012)

In vitro studies are the initial step in preclinical testing and provide valuable insights into a drug's behaviour at the cellular and molecular level. They are conducted in a controlled laboratory environment using isolated cells, tissues, or biological molecules. Researchers investigate how the drug candidate interacts with specific biological targets, such as proteins or enzymes. These studies help identify potential mechanisms of action and therapeutic effects.

While In vivo studies involve administering the drug candidate to animals to assess its safety, effectiveness, and pharmacological properties. Common animal models include rodents (mice and rats), rabbits, dogs, and non-human primates. Researchers evaluate pharmacokinetics (absorption, distribution, metabolism, and excretion) in living organisms.(Karen L steinmetz, 2009). It also assess potential toxicities, including side effects and adverse reactions. These studies provide information on the drug's impact on the physiology, behaviour, and organ systems of the animals. In vivo testing helps bridge the gap between laboratory results and potential human responses to the drug. On the other hand Toxicology studies involve administering the drug candidate at varying doses to establish the dose-response relationship. It aims to determine the drug candidate's safety profile and assess potential side effects and adverse reactions. Researchers investigate the drug's effects on vital organs and systems to identify potential toxicities.

As Preclinical testing assesses how the drug behaves within the body, including its ADME properties (absorption, distribution, metabolism, and excretion). Pharmacodynamics studies examine the drug's effects on the body and mechanisms of action.Regulatory agencies, such as the FDA (in the United States) or the EMA (in Europe), provide strict guidelines for preclinical testing. Data generated during preclinical studies are submitted as part of the regulatory application, such as the Investigational New Drug (IND) application in the U.S.

Ethical considerations are paramount in animal testing. Researchers must follow ethical guidelines to minimize animal suffering and ensure humane treatment. Efforts are made to reduce or replace animal testing with alternative methods, such as computer modeling and cell-based assays. Preclinical data analysis guides decisions on whether a drug advances to human trials. Positive findings on safety and efficacy support progression. However, if significant safety concerns emerge during preclinical testing, further development may be paused or modified.

This cautious approach ensures the safety of future trial participants and the drug's overall success.

Investigational New Drug (IND) application:

An Investigational New Drug (IND) Application is a formal petition submitted to the U.S. Food and Drug Administration (FDA) by either a pharmaceutical corporation or a researcher, seeking authorization to carry out human clinical trials for a novel medication. The application comprises information derived from preliminary laboratory investigations, specifics about the intended clinical trials, manufacturing particulars, and safety documentation. The FDA assesses the IND to ascertain the safety and scientific validity of the proposed trials prior to granting permission for their initiation.

Under current federal regulations, a drug must possess an approved marketing application before it can be transported or distributed across state lines. Given that a sponsor often intends to ship the investigational drug to clinical investigators in multiple states, they must seek an exemption from this legal requirement. The IND serves as the formal mechanism through which the sponsor secures this exemption from the FDA.

Several crucial sections within the IND include the clinical protocol, encompassing suitable subject eligibility, inclusion/exclusion criteria, investigational endpoints, follow-up intervals, cessation criteria, and dosage rationale (initiation dose/escalation). This also comprises dosing frequency and timetables, process and qualification studies, as well as safety, pharmacology, and toxicology investigations. In the event of a hold on the clinical trial application, the applicant can request a clinical trial restart by resubmitting the IND registration materials following the completion of supplementary research.(Zhao, 2021)

During the initial stages of preclinical development for a new drug, the sponsor's primary objective is to determine if the product is reasonably safe for initial human use and if the compound exhibits pharmacological activity that justifies its progression into commercial development. When a product is identified as a promising candidate for further development, the sponsor then focuses on gathering the necessary data and information to establish that the product does not pose unreasonable risks when used in limited, early-stage clinical trials. The FDA's involvement in the new drug's development commences when the drug's sponsor (typically the manufacturer or potential marketer), after conducting preliminary assessments of the new molecule for pharmacological activity and acute toxicity potential in animals, expresses the intention to assess its diagnostic or therapeutic potential in humans. At this juncture, the legal status of the molecule shifts under the Federal Food, Drug, and Cosmetic Act, marking it as a new drug subject to the specific requirements of the drug regulatory system.

Submitting an IND involves completing three sets of forms: one outlining the study (FDA Form 1571), another providing details about the investigator and study site (FDA Form 1572), and a third certifying the study's registration in the national clinical trials database (FDA Form 3674). Once the FDA acknowledges receipt and assigns an IND, the study can commence 30 days later if approved. If the FDA requires additional information or places the study on a

"clinical hold," it cannot proceed. Throughout the active IND period, the investigator must adhere to regulations for study monitoring and reporting to the FDA. (MEB, 2009)

Following the submission of the IND, the sponsor is required to observe a waiting period of 30 calendar days before commencing any clinical trials. During this interval, the FDA has the opportunity to scrutinize the IND for safety to ensure that research participants will not be exposed to unjustifiable risks.

Clinical trials:

Clinical trials follow a structured process involving specific procedures to rigorously assess the safety and effectiveness of medical treatments or drugs. These procedures include protocol development, participant recruitment, and obtaining informed consent. Randomization(EM Beller, 2002), baseline assessments, data collection, and adverse event monitoring are integral steps. Statistical analysis and predefined endpoints are used to evaluate treatment effectiveness and safety. Blinding and double-blinding techniques minimize bias, while interim analyses and oversight by independent data monitoring boards ensure trial integrity. Ultimately, the trial's results are reported to inform medical practice and regulatory decisions, guiding future procedures in healthcare research.

Phase 1 – Safety Assessment: This initial phase involves a small group of volunteers, often healthy individuals are given with the drug. The primary objective is to assess the drug's safety, including dosage levels and potential side effects. Researchers closely monitor participants and gather data on how the drug behaves within the body.

Phase 2 – Efficacy and Side Effects: In Phase 2, a larger group of patients who have the targeted medical condition participate. The focus shifts to evaluating the drug's effectiveness in treating the specific condition. Researchers continue to monitor for side effects and assess whether the drug demonstrates therapeutic benefits.

After the completion of Phase 3, all the accumulated data is submitted to regulatory agencies such as the U.S. Food and Drug Administration (FDA).(Dickersin, 2003) At this stage, regulatory experts meticulously examine the drug's safety, efficacy, and overall quality. If the drug satisfies the stringent requirements and demonstrates its safety and effectiveness when used as directed, it receives regulatory approval for market use. This approval marks a significant milestone in the drug development process, signifying that it has met the necessary standards. Following regulatory approval, Phase 4 comes into play. This phase involves continuous monitoring of the drug's performance in real-world clinical practice. Its primary focus is to detect any rare or long-term side effects that may not have been apparent during earlier phases. By collecting and analyzing data from patients using the drug, Phase 4 ensures that it remains safe and effective as it becomes more widely available. Any emerging safety concerns during this phase can prompt further actions, including updates to labeling or issuing warnings to healthcare professionals and patients.

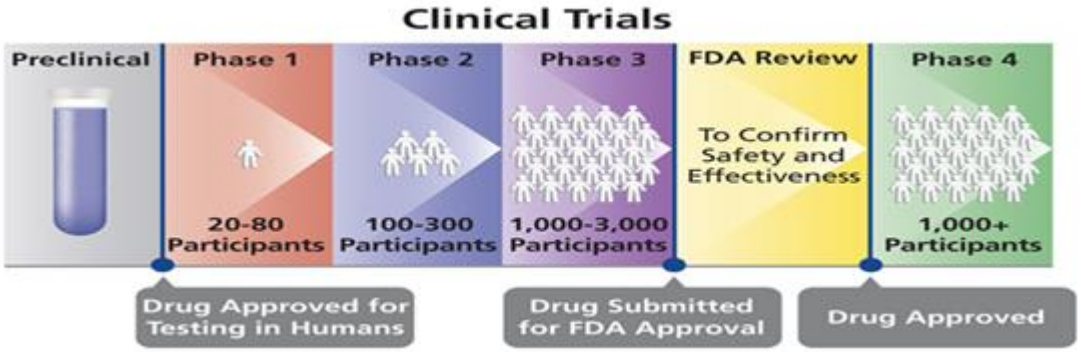

Fig. 1: Clinical trials (credits: onlinesciencenotes.com)

New Drug Application (NDA) submission:

The NDA application serves as the formal submission in which pharmaceutical sponsors formally request FDA approval for the sale and marketing of a new pharmaceutical in the United States. The information collected from animal studies and human clinical trials conducted during the Investigational New Drug (IND) phase is incorporated into the NDA.

In the United States, drug products can gain FDA approval through three primary regulatory pathways: 505(b)(1) and 505(b)(2) New Drug Applications (NDAs) and 505(j) Abbreviated NDAs (ANDAs). The 505(b)(2) NDA pathway is particularly relevant when leveraging information from an existing approved drug (listed drug or LD) to support the development of a new drug. This pathway was created to avoid unnecessary duplication of studies, including nonclinical research, already conducted on the LD.In contrast, generic drugs typically follow the 505(j) ANDA pathway, relying on safety and efficacy data from the reference listed drug (RLD) and demonstrating bioequivalence. The 505(b)(1) NDA pathway involves sponsor-conducted studies and extensive resources but offers market exclusivity advantages. The 505(b)(2) NDA pathway provides potential benefits, including reduced nonclinical and clinical requirements compared to 505(b)(1) NDAs. However, it comes with challenges, such as accelerated CMC programs, competition from other companies, and considerations related to LD patents and exclusivity. Overall, the 505(b)(2) NDA pathway allows applicants to reference published literature and FDA findings to meet registration requirements, potentially reducing the need for extensive nonclinical testing. Early communication with the FDA is crucial to outline and obtain feedback on the nonclinical aspects of a 505(b)(2) NDA program.(William F. Salminen, Marc E. Wiles, Ruth E. Stevens, 2019)

NDAs must adhere to the specified format and include the pertinent information required by this section, tailored to the specific submission. The submission typically involves the provision of three copies of the NDA, which include an archival copy, field copy a review copy. In the case of an NDA for a new drug, it typically comprises an application form, an index, summary, five to six technical sections, patient data presented in case report tabulations, case report forms, drug samples, which may include a Medication Guide if applicable.

New Drug Applications (NDAs) encompass a comprehensive compilation of data that extends beyond the contents of an Investigational New Drug (IND) application. In addition to the

information included in the IND, NDAs contain critical data from phases I to III of clinical trials. These trials provide substantial evidence of the drug's safety and effectiveness in humans. The approval process for a new drug is a multifaceted and intricate journey. The NDA submission marks the final major obstacle to clear before a drug can gain market entry. At this stage, regulatory authorities meticulously evaluate the NDA to determine whether the drug meets stringent standards for safety, efficacy, and quality. Approval signifies that the drug is deemed suitable for widespread use by the general population, making it a pivotal milestone in the drug development process. (Sandeep Murthy, Shashidhara Murthy, 2023)

The NDA aims to furnish sufficient data for FDA evaluators to make critical determinations, including assessing the drug's safety and efficacy in its intended applications, ascertaining the appropriateness of the proposed labeling, and ensuring the adequacy of manufacturing methods and quality controls to maintain the drug's integrity, potency, quality, and purity.

The ultimate objective of drug development is to obtain approval for a new drug. After compiling data from preclinical and clinical trials, a New Drug Application (NDA) must be presented to the regulatory body for endorsement. While there are commonalities in submission requirements worldwide, historically, these applications have diverged. Regulatory authorities operating within the framework of the International Conference on Harmonisation (ICH) aspire to streamline the application process by introducing the Common Technical Document, aiming to simplify the procedure for applicants. (Molzon, J, 2003)

Regulatory review:

The regulatory review of a drug is a meticulous and multi-faceted process that occurs after the completion of Phase 3 clinical trials. At this stage, all the data collected during the drug’s development, including safety and efficacy results, are compiled into a comprehensive dossier. This dossier, often known as a New Drug Application (NDA) in the United States or a Marketing Authorization Application (MAA) in Europe, is submitted to the relevant regulatory agency responsible for drug approval, such as the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA).(Dziurzynski, 1996)

Upon submission, the regulatory agency forms a team of experts encompassing various fields, including pharmacology, toxicology, clinical research, and statistics. This multidisciplinary team undertakes a thorough review of the submitted data, aiming to evaluate all aspects of the drug’s development.They delve into the safety data, meticulously assessing adverse events, potential side effects, and any associated risks. Simultaneously, they scrutinize efficacy data to gauge how effectively the drug treats the targeted medical condition and whether its benefits outweigh any identified risks. Quality control measures are examined closely, ensuring the drug’s consistency and reliability in terms of manufacturing processes.

The regulatory review process extends to the pharmacokinetics of the drug, analyzing how It is absorbed, distributed, metabolized, and excreted by the human body. Comprehensive evaluations are also conducted on preclinical testing results and data from all phases of clinical trials. Critical to this process is the determination of appropriate labelling for the drug, including

specifying approved indications, dosage instructions, potential side effects, contraindications, and warnings. Central to the review is the benefit-risk assessment, wherein regulatory experts weigh the therapeutic benefits of the drug against its potential risks. This assessment ensures that the drug offers a net positive effect on patient health.

In some instances, regulatory agencies may convene expert advisory committees composed of independent healthcare professionals and scientists. These committees provide additional insights and recommendations concerning the drug's approval. Throughout the review process, there is a continuous dialogue between regulatory agencies and the drug developer. Questions, requests for additional data, and clarifications are common occurrences during this phase.

Ultimately, once the comprehensive review is complete, the regulatory agency makes a decision regarding the drug's approval. If the drug is found to be safe and effective, with its benefits outweighing its risks, it receives regulatory approval for market use. This signifies that the drug meets the stringent standards required to ensure patient safety and efficacy, marking a significant milestone in its journey toward becoming accessible to healthcare professionals and patients.

FDA approval (or equivalent in other countries):

The drug approval process of the U.S. Food and Drug Administration is designed to offer consumers confidence that a drug, once available in the marketplace, is safe and effective for its intended purpose. Introducing a drug to the market typically requires an average of 12-year. Nonetheless, there are apprehensions that the processes of the Food and Drug Administration may not entirely fulfil the intended objectives of ensuring safety and efficacy.(Norman, 2016)

FDA drug approval involves a rigorous evaluation process where the agency assesses a drug's effects to ensure that its benefits outweigh potential risks for the intended patient group. This process includes analyzing the targeted medical condition, existing treatments, and clinical data submitted by the drug manufacturer. Risk management strategies, such as FDA-approved labels and, if needed, comprehensive Risk Management and Mitigation Strategies (REMS), are developed. The FDA's assessments may involve complexities and uncertainties, but they rely on the best available scientific data. In some cases, expedited approval is granted for drugs addressing serious or life-threatening conditions, potentially based on surrogate endpoints. Post-marketing trials are required to validate the drug's actual benefit, with approval withdrawal possible if verification fails.

The FDA's evaluation of new drug applications aligns with the Federal Food, Drug, and Cosmetic Act, which stipulates the need for "adequate and well-controlled investigations" to establish efficacy. FDA guidance suggests that drug manufacturers should submit a minimum of two trials, termed "pivotal" efficacy trials, each providing independent evidence of effectiveness. Nonetheless, the guidelines acknowledge flexibility, considering situations where a single efficacy trial may suffice for approval. Furthermore, in specific scenarios, the FDA issues written directives on the design of pivotal efficacy trials, covering elements like sample selection and comparator choice. Additionally, the agency may offer further guidance during sponsor

meetings. For instance, in the case of drugs assessed under the accelerated approval pathway, designed to expedite treatments for life-threatening conditions, pivotal efficacy trials may employ surrogate endpoints that reasonably predict clinical benefit.

The clinical research findings available at the time of a drug's approval hold significant importance. These findings, if disclosed, serve as the sole source of information for patients and physicians when making decisions about using a newly approved medication. However, adaptable approval standards can result in FDA approval based on varying numbers and the robustness of clinical trials, leading to different levels of confidence regarding the risks and benefits of newly approved pharmacologic and biologic agents. Therefore, our objective was to systematically evaluate this issue, assessing the strength of clinical trial evidence supporting FDA approval decisions for innovative therapeutic agents, both pharmacologic and biologic, from 2005 to 2012. This evaluation entailed characterizing pivotal efficacy trials, including aspects such as their size, design, duration, and endpoints.(Downing NS, Aminawung JA, Shah ND, Krumholz HM, Ross JS, 2013)

Post marketing surveillance:

Post-marketing surveillance, often referred to as pharmacovigilance, is a crucial phase in the life cycle of pharmaceutical products and medical devices. It takes place after regulatory approval when these products are available to the wider public. Unlike controlled clinical trials, post-marketing surveillance observes how these treatments perform in the real world, encompassing a diverse patient population with varying health conditions.

One of its primary purposes is to detect rare or unexpected side effects that might not have surfaced during the controlled environment of clinical trials. While clinical trials are essential for establishing a treatment’s safety and efficacy, they typically involve a relatively small and carefully selected group of participants. Post-marketing surveillance, on the other hand, extends its reach to thousands or even millions of patients, providing a more comprehensive view of how the treatment affects people.

Healthcare professionals, patients, and manufacturers play pivotal roles in this surveillance system. They are encouraged to report any adverse events, unexpected side effects, or concerns related to the use of pharmaceutical products and medical devices. These reports contribute to a growing and dynamic database of safety information.(alomar, 2020)

Regulatory authorities closely analyze the data collected through post-marketing surveillance. Their objective is to identify potential safety signals, which are patterns or trends suggesting new or previously unrecognized safety concerns. When a safety signal emerges, it triggers a thorough investigation. This investigation may lead to regulatory actions, such as updating product labeling to include warnings or precautions, issuing safety alerts, or in rare cases, withdrawing the product from the market.

Ultimately, the core purpose of post-marketing surveillance is to enhance patient safety. By continuously collecting and analyzing safety data in real-world scenarios, it ensures that pharmaceutical products and medical devices remain safe and effective for those who use them.

This ongoing monitoring and regulatory responsiveness help protect public health and provide valuable insights for healthcare professionals and patients.

Fig. 1: Post market surveillance (credits: ArborMetrix)

Manufacturing and distribution:

Crucial interconnections exist among the efficiency of pharmaceutical manufacturing, drug pricing, and public health in the United States. These connections, as recent research from both academics and the US Food and Drug Administration indicates substantial opportunities to enhance pharmaceutical manufacturing processes. It presents two models that assess the consequences of reduced manufacturing expenses on pharmaceutical prices and corporate profits. These models effectively set the parameters for potential future advantages stemming from improved manufacturing efficiency. They estimate, for instance, that a 30% reduction in manufacturing costs could yield social value ranging from $1.0 to $12.3 trillion for the United States.(Vernon JA, Hughen WK, Trujillo AJ, 2007)

Pharmaceutical manufacturing encompasses the large-scale production of medicinal compounds, primarily executed by pharmaceutical enterprises. This intricate procedure comprises a succession of discrete unit operations, including milling, granulation, coating, and tablet pressing, among others. In continuous manufacturing, a consistent supply of raw materials and energy is maintained within the system, concomitant with the ongoing extraction of finished products. The efficacy of this process is contingent upon the steadfastness of material flowrates. The pharmaceutical sector adheres rigorously to stringent prerequisites and manufacturing protocols, mandating that pharmaceutical production equipment align impeccably with good manufacturing practices (GMP). This adherence is indispensable for assuring the caliber and safety of the pharmaceuticals manufactured.

The process of drug distribution within markets is a sophisticated and intricately designed supply chain operation aimed at ensuring the efficient and secure delivery of pharmaceutical products to patients, healthcare facilities, and pharmacies. It typically commences with the manufacturing of drugs, followed by their distribution. Pharmaceutical wholesalers play a pivotal role in this phase as they acquire medications and distribute them to a diverse network of retail pharmacies, hospitals, and healthcare providers. Patients receive these medications either directly from local pharmacies or within healthcare settings where they are administered. Additionally, the landscape of drug distribution has evolved with the emergence of online and specialized pharmacies, which provide patients with convenient access to medications, particularly those of a

specialized or prescription nature. Overall, the essence of drug distribution within markets lies in its collaborative endeavor to facilitate access to essential medications while steadfastly upholding stringent quality and safety standards.

Variability among patients poses a significant challenge when administering medications, particularly considering diverse factors like health conditions, pharmacokinetics, age, fitness, gender, and race. Addressing this challenge calls for the implementation of intelligent and personalized drug delivery systems featuring controlled release profiles, all of which can be achieved through innovative approaches to manufacturing. Additive manufacturing (AM) presents a host of opportunities, including complete customization, design flexibility, on-site production, and material recycling. Consequently, there has been a consistent rise in both academic and industrial interest in utilizing additive manufacturing for drug delivery, yielding impressive outcomes across a wide spectrum of products. Additive manufacturing (AM), a crucial component of digital technology, has made significant advancements over the past three decades. This manufacturing method was originally conceived by Charles Hull in 1986, employing UV-sensitive polymers and ultraviolet (UV) light to produce three-dimensional objects (Prasad and Smyth, 2016). Initially known as Stereolithography Apparatus (SLA), this technology has since undergone continuous evolution. Researchers and engineers have devised numerous pioneering AM techniques. Presently, a wide array of commercially available AM equipment exists, with many more innovative concepts still undergoing exploration and development. (Abdullah Mohammed, Amr Elshaer, Pooya Sareh, Mahmoud Elsayed, Hany Hassanin, 2020)

Market launch:

The market launch of a new drug is a pivotal moment in the pharmaceutical industry, marking the culmination of years of research, development, and regulatory approval. This process involves a meticulously planned and coordinated effort to introduce the medication to healthcare providers and patients. It begins with the drug receiving regulatory approval from agencies like the U.S. Food and Drug Administration (FDA) or the European Medicines Agency (EMA). This approval signifies that the drug has met stringent standards for safety, efficacy, and quality.

With regulatory approval in hand, drug manufacturers initiate large-scale production to meet anticipated demand. Stringent quality control measures and adherence to Good Manufacturing Practices (GMP) ensure that each dose is consistent and of high quality. A critical aspect of the market launch is the development of a comprehensive marketing strategy. This strategy encompasses identifying the target patient population, understanding the competitive landscape, and crafting key messaging and branding for the drug. Pricing strategies are carefully determined, considering production costs, market competition, and reimbursement considerations. Negotiations with payers, such as insurance companies or government healthcare programs, may be necessary to secure favorable coverage and reimbursement terms. A dedicated sales force is assembled to promote the drug to healthcare providers, including physicians,

nurses, and pharmacists. Distribution channels are established to ensure efficient delivery to pharmacies, hospitals, and clinics.

Education is paramount. Healthcare professionals need to be well-informed about the drug's indications, dosages, potential side effects, and patient selection criteria. This often involves organizing medical conferences, providing educational materials, and conducting training programs. Marketing materials, including brochures, websites, and advertising campaigns, are created to raise awareness about the drug among healthcare providers and patients. Ensuring patient access to the drug is essential. This involves navigating complex healthcare systems, securing formulary inclusion, and addressing any prior authorization requirements.

Even after the launch, the drug's safety and efficacy continue to be monitored through post-marketing surveillance.(Bate, 2008) This ongoing vigilance helps identify any previously unrecognized side effects or safety concerns. Lifecycle management is a consideration too. Pharmaceutical companies may explore additional indications or formulations for the drug to expand its market and extend its lifecycle.

Feedback from healthcare providers and patients is continuously gathered to understand the real-world performance of the drug and make necessary adjustments in marketing and product development.

In summary, the market launch of a drug is a multifaceted endeavour that requires the collaboration of various departments within pharmaceutical companies. Effective communication, education, market access, and post-marketing surveillance are key elements in ensuring that a new drug reaches the patients who can benefit from it while upholding the highest standards of safety and efficacy.

Lifecycle management:

In recent years, there has been a conspicuous deceleration in the advancement of new molecular entities (NMEs). An analysis of FDA-approved drugs spanning the period from 1950 to 2008 highlights a reduction in the number of sanctioned NMEs, particularly evident since reaching a pinnacle in 1996. Furthermore, the expenses associated with ushering an NME into the market have consistently surged, escalating at an annual rate of 13.4% since the 1950s. In this demanding landscape, the concept of lifecycle management (LCM) takes on renewed significance, emerging as a pivotal strategy to enhance the efficiency of investments in drug discovery. Drug lifecycle management (LCM) hinges on two critical elements: patent protection and market exclusivity, both granted by national pharmaceutical regulatory authorities.

Market exclusivity, primarily designed to provide developers with exclusivity in the USA and Japan, differs significantly between these countries in terms of their patent term extension systems. While it is widely accepted that the individual systems in each country influence drug LCM, there remains a gap in the literature concerning comparative analyses of drug LCM in the USA and Japan, with a specific focus on patent term extension. Therefore, this article's background section underscores prior research related to the fundamental strategies employed in drug LCM. Subsequently, we conduct a case study that compares the patent term extension

systems and drug LCM practices in both the United States and Japan. (Takayuki Yamanaka, Shingo Kano, 2016)

Life cycle management (LCM) entails strategic planning for a drug's market positioning, spanning from pre-regulatory approval through patent expiration or loss of exclusivity. It encompasses preparations for follow-up studies post-initial approval and potential label expansions. In the realm of LCM, pricing also assumes significance. Price adjustments tend to be more conservative during the initial stages of a drug's life cycle when the emphasis lies on market growth and adoption. Conversely, as a drug approaches competition from generics or biosimilars, price hikes may become more prevalent. In alignment with this, substantial capital investments, such as new randomized clinical trials, are more likely during the early stages, while investments may decrease as the long-term prospects dwindle over time.

Evidence-based research conducted during the post-marketing stage to assess a product's safety, efficacy, and overall development is a vital component of comprehensive lifecycle management (LCM) within the medical field. In medical practice, various LCM approaches are typically employed, including the execution of new branding strategies, repositioning products to address new indications, creating combination drugs, developing innovative product formulations, tailoring products for pediatric use, and augmenting clinical utility to provide additional value .Despite some reports discussing how product-related research contributes to LCM, there remains a gap in understanding the complete landscape of LCM within the real-world marketplace. Furthermore, the assessment of the impacts of LCM activities on generating additional value has not been sufficiently evaluated. This highlights the need for comprehensive analyses of LCM strategies and their effects in the medical field. (Yukiko Hashitera, Chikako Saotome, Hirokazu Yamamoto, 2013)

Patent protection and generic entry:

Patent protection is a fundamental mechanism in the pharmaceutical industry that encourages innovation. When a pharmaceutical company develops a new drug, it is granted a patent, typically for a period of 20 years from the date of filing. During this period, the company has exclusive rights to manufacture and sell the drug. This exclusivity serves as a financial incentive, allowing the company to set prices that enable them to recover the substantial costs of drug development, including research, clinical trials, and regulatory approval. The flip side of patent protection Is that it leads to higher drug prices during the patent-protected period. Patients often pay more for brand-name drugs because there is no competition. However, when the patent expires, the pharmaceutical market opens up to generic entry.(Huskcamp, 2008)

Generic entry is a process where other pharmaceutical companies can manufacture and market generic versions of the drug. These generic drugs have the same active ingredients, strength, dosage form, and route of administration as the brand-name drug. They are also required to demonstrate bioequivalence, meaning they produce the same therapeutic effect in the body as the original drug.

The Introduction of generic drugs into the market can significantly impact healthcare costs. Generic drugs are typically more affordable than their brand-name counterparts, making

essential medications more accessible to patients. The competition among generic manufacturers can further drive down prices. Additionally, generic entry allows healthcare providers to consider therapeutic interchange, where they may switch patients from a brand-name drug to its generic equivalent to reduce costs while maintaining the same therapeutic benefits. Over time, generic drugs often gain a substantial market share, especially when multiple generic manufacturers produce the same drug. This competition not only benefits patients by lowering drug prices but also encourages continued innovation in the pharmaceutical industry. Pharmaceutical companies strive to develop new, patent-protected drugs to maintain profitability, driving advancements in healthcare.(Frank, 2004)

Patent protection and generic entry are essential elements in the pharmaceutical industry. Patents incentivize innovation, while generic entry introduces competition that can reduce drug prices and improve access to medications, striking a balance between fostering innovation and ensuring affordability in healthcare.

Fig. 3: Symbol of Patent (credits: IndiaFillings)

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CATALYSING CLEANUP: EXPLORING HYDROLASES' VITAL ROLE IN POLLUTANT BIODEGRADATION

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Abstract:

In the realm of environmental sustainability, the search for effective strategies to combat pollution has led to a deeper appreciation for the role of enzymatic biodegradation. Among the diverse array of enzymes that participate in this natural remediation process, hydrolases emerge as powerful catalysts for breaking down a wide range of pollutants. This book chapter delves into the pivotal role of hydrolases in the biodegradation of pollutants, shedding light on their catalytic prowess and the environmental benefits they offer. As versatile enzymes, hydrolases operate through hydrolysis reactions, which involve the cleavage of chemical bonds with the incorporation of water molecules. Through this mechanism, they exhibit specificity for various substrates, enabling them to target pollutants such as esters, lipids, proteins, and carbohydrates. This specificity, combined with the diversity of hydrolase classes, equips them to participate in intricate enzymatic pathways that facilitate pollutant breakdown. From industrial wastewater treatment to soil and aquatic ecosystem remediation, hydrolases have proven to be valuable tools in the arsenal against pollution. The incorporation of hydrolase-producing microorganisms in bioremediation strategies demonstrates their capability to collaborate within microbial consortia, effectively degrading complex pollutants. Furthermore, recent advancements in genetic engineering have allowed for the tailored overexpression of hydrolases, paving the way for enhanced pollutant removal and the optimization of bioremediation processes. By exploring recent research, case studies, and emerging trends, this study underscores the pivotal role that hydrolases play in catalysing cleanup efforts and underscores the need for continued research to harness their full potential in the quest for a cleaner and healthier planet.

Introduction:

In an era defined by mounting environmental challenges and an urgent need for sustainable solutions, the science of biodegradation has emerged as a beacon of hope for restoring ecological balance [1]. Amid this pivotal endeavour, the role of enzymes in catalysing the breakdown of pollutants has garnered significant attention [2]. Among these biocatalysts, hydrolases, with their remarkable versatility and specificity, stand out as essential players in the intricate web of biodegradation processes [3]. This book chapter embarks on a journey into the captivating world of hydrolases, unveiling their vital role in driving the cleanup of pollutants through biodegradation. From the smallest ester bond to the complex carbohydrates that shape our environment, hydrolases possess an unparalleled ability to cleave these chemical bonds, facilitated by the addition of water molecules [4]. This enzymatic hydrolysis, seemingly simple yet profoundly impactful, forms the cornerstone of hydrolases' exceptional prowess in pollutant degradation. Through this chapter, we will uncover the diversity within the realm of hydrolases,

from lipases and esterases to glycosidases and beyond, each with a distinct set of capabilities that render them uniquely suited to target specific pollutant classes. As we peer into the world of enzymatic pathways, we will witness hydrolases' role in orchestrating intricate chemical choreographies, guiding pollutants toward transformation into environmentally benign compounds. The applications of hydrolases are far-reaching, spanning from industrial wastewater treatment and soil restoration to the revival of aquatic ecosystems tainted by contamination. The remarkable journey into hydrolases' world does not stop at their natural functions. Genetic engineering, a testament to human ingenuity, has unlocked the potential to manipulate microorganisms to produce enhanced hydrolases tailored to specific pollutants [5]. Amidst these challenges and triumphs, the overarching objective remains clear: to harness the extraordinary capabilities of hydrolases in transforming pollution into renewal. The remarkable synergy between enzymatic prowess and human ingenuity stands poised to redefine our approach to environmental restoration, underpinned by the collaboration of science, technology, and the very forces of nature that have shaped our world.

1. The hydrolase symphony: An overview

Enzymes, the molecular architects of life, orchestrate a symphony of biochemical reactions that underpin the intricate processes within living organisms [6]. Among these biochemical virtuosos, hydrolases emerge as a diverse and indispensable group, endowed with the remarkable ability to catalyze hydrolysis reactions. Hydrolysis, the process by which water molecules cleave chemical bonds, is a fundamental mechanism that holds the key to unlocking energy, breaking down complex molecules, and transforming substances in both biological and environmental contexts [7]. As catalysts of hydrolysis, hydrolases engage in reactions that span from the simple hydrolysis of ester bonds to the complex breakdown of polysaccharides and lipids [8]. Their unique ability to accelerate reactions that might otherwise occur at impractically slow rates underpins a vast array of biological and biochemical processes. Hydrolases are united by their shared catalytic function, but their diversity is striking. This group encompasses a rich spectrum of enzymes, each evolved to recognize specific chemical substrates and catalyze reactions with exquisite precision. Lipases target lipids, esterases hydrolyze ester bonds, proteases dismantle proteins, and glycosidases cleave glycosidic linkages in carbohydrates. [9-12]. These specialized enzymes play a pivotal role in shaping the intricate biochemical pathways that sustain life. The versatility of hydrolases extends beyond biological realms. In the arena of environmental remediation, these enzymes prove to be valuable allies in breaking down pollutants and mitigating ecological harm. Through hydrolysis, they facilitate the degradation of recalcitrant compounds, contributing to the restoration of polluted ecosystems. This very characteristic underscores the profound impact that hydrolases have in addressing some of our planet's most pressing challenges [13].

2. Highlighting the versatility of hydrolases in targeting various classes of pollutants

The versatility of hydrolases is a remarkable attribute that equips these enzymes to effectively target and degrade various classes of pollutants [14]. Their ability to recognize specific chemical bonds and catalyze hydrolysis reactions makes them invaluable tools in the cleanup of diverse environmental contaminants. Here, we highlight the remarkable versatility of hydrolases in addressing different classes of pollutants:

2.1 Lipids and Fats: Lipases are hydrolases specifically tailored to target lipids and fats. In environments contaminated with oil spills or industrial discharge, lipases play a critical role in breaking down complex lipid molecules into simpler fatty acids and glycerol. This enables the conversion of non-degradable oils into biodegradable components, facilitating the remediation of aquatic and terrestrial ecosystems [15].

2.2 Esters and Ethers: Esterases are hydrolases that excel at cleaving ester bonds, a common linkage in a range of synthetic compounds, including pesticides and plasticizers. By catalyzing the hydrolysis of these esters, esterases contribute to the degradation of harmful chemicals, reducing their persistence and potential harm to the environment [16].

2.3 Proteins and Peptides: Proteases, another class of hydrolases, specialize in breaking down proteins and peptides into amino acids. This ability is valuable in scenarios where organic waste or pollutants like detergents and certain industrial byproducts contain proteinaceous material. Proteases aid in degrading these materials into their fundamental building blocks [17].

2.4 Carbohydrates: Glycosidases are hydrolases that target glycosidic linkages in carbohydrates. These enzymes play a role in breaking down complex carbohydrates like cellulose and chitin found in plant matter and the exoskeletons of insects. By degrading these components, glycosidases contribute to the recycling of organic material and the prevention of biomass accumulation [18].

2.5 Nucleic Acids: Nucleases are hydrolases that target nucleic acids, including DNA and RNA. These enzymes find applications in bioremediation scenarios involving genetic material, such as the breakdown of genetic pollutants released from genetically modified organisms or medical waste [19].

3. From lipids to esters: Hydrolases' substrate specificity

Hydrolases, a diverse group of enzymes renowned for their catalytic prowess, exhibit a fascinating attribute that sets them apart: their remarkable substrate specificity. This inherent capability allows hydrolases to selectively target and cleave specific chemical bonds within a wide range of substrates, spanning from lipids to esters and beyond [20-21]. This chapter delves into the intricate world of hydrolases' substrate specificity, shedding light on their ability to engage with diverse molecules and contribute to various biological and environmental processes.

3.1 Understanding substrate specificity:

At the core of hydrolases' substrate specificity lies their intricately shaped active sites. These molecular pockets are precisely contoured to accommodate particular substrate molecules, holding them in place for catalytic reactions [22]. As a result, each type of hydrolase is finely tuned to recognize a distinct class of molecules, often defined by the specific chemical bonds they contain.

3.2 Lipases: Masters of lipid degradation

Lipases, a prominent subclass of hydrolases, exemplify the concept of substrate specificity. These enzymes target lipids—fatty molecules essential for energy storage and cellular structure. By binding to lipid molecules at specific points, lipases efficiently break the fatty acid chains away from glycerol backbones [23]. This process, called hydrolysis, yields fatty acids and glycerol, which can be further metabolized or incorporated into cellular structures.

3.3 Esterases: Precision in breaking esters

Esterases, another subset of hydrolases, excel in cleaving ester bonds—a crucial linkage present in various synthetic and natural molecules. Esterases' substrate specificity enables them to act on compounds such as pesticides, plasticizers, and fragrance components [24]. By breaking ester bonds, these enzymes contribute to the degradation of pollutants and the recycling of organic materials in the environment.

3.4 Proteases and glycosidases: Enzymatic diversity

Proteases, which target proteins, and glycosidases, specialized in cleaving glycosidic linkages in carbohydrates, showcase the diversity of hydrolases' substrate preferences. Proteases break down proteins into their constituent amino acids, aiding in nutrient recycling and cellular processes [25]. Glycosidases, on the other hand, act on complex carbohydrates like cellulose, enabling the breakdown of plant matter and promoting ecosystem nutrient cycles.

3.5 Environmental applications

Hydrolases' substrate specificity finds practical applications in environmental bioremediation efforts. By harnessing their ability to recognize and act on specific pollutants, researchers can engineer hydrolases to efficiently degrade contaminants like oils, plastics, and industrial chemicals [26]. These tailored enzymes contribute to the restoration of polluted environments and the reduction of ecological harm.

4. Examples of lipases degrading fats, esterase breaking down esters, and more:

4.1 Lipases degrading fats:

4.1.1 Oil spill remediation: After an oil spill, large amounts of hydrophobic fats and oils coat water surfaces, posing a significant threat to aquatic ecosystems. Lipases play a crucial role in breaking down these complex lipids into simpler fatty acids and glycerol. For instance, during the Exxon Valdez oil spill in 1989, naturally occurring marine bacteria produced lipases that aided in the degradation of spilled oil [27].

4.1.2 Waste water treatment: Lipases are employed in wastewater treatment plants to break down fats, oils, and grease that accumulate in sewage systems. These enzymes convert these substances into more manageable components, reducing the risk of clogged pipes and environmental contamination [28].

4.2 Esterase breaking down esters:

4.2.1 Biodegradable plastics: Esterase are crucial in the degradation of biodegradable plastics made from ester-linked polymers. These plastics, designed to break down more rapidly in the environment, rely on esterases to cleave the ester bonds, ultimately leading to their decomposition into harmless fragments [29].

4.2.2 Pesticide detoxification: Many pesticides used in agriculture and industry contain ester bonds that can be targeted by esterases. These enzymes contribute to the detoxification of pesticides in the environment, breaking them down into less harmful compounds [30].

4.3 Glycosidases breaking down carbohydrates:

4.3.1 Cellulose degradation: Glycosidases are vital in breaking down cellulose, a major component of plant cell walls. Microorganisms, including bacteria and fungi, produce glycosidases that hydrolyse the glycosidic linkages in cellulose, converting it into glucose and other simple sugars that can be utilized as a carbon source [31].

4.3.2 Chitin decomposition: Chitin is a polymer found in the exoskeletons of insects and the cell walls of fungi. Glycosidases play a role in breaking down chitin into its monomeric components, aiding in the recycling of insect remains and contributing to nutrient cycling in ecosystems [32].

4.4 Nutrient recycling: Proteases are involved in the digestion of proteins in the stomachs of animals, breaking them down into smaller peptides and amino acids that can be absorbed and used for various cellular processes, including protein synthesis [33].

4.5 Decomposition of organic matter: In soil ecosystems, proteases contribute to the breakdown of organic matter by cleaving peptide bonds within proteins. This process releases amino acids, which serve as an essential nutrient source for plants and microorganisms [34].

5. Case studies illustrating the role of hydrolases in complex biodegradation processes

The intricate role of hydrolases in biodegradation extends far beyond simple reactions. These enzymes play a pivotal role in complex, real-world scenarios, where pollutants are transformed through multi-step pathways [35]. The following case studies provide tangible examples of how hydrolases contribute to the cleanup of diverse environmental contaminants.

Case Study	Pollutant	Hydrolase Involvement	Process
Oil Spill Remediation	Crude oil spilled into marine environments	Lipases	Lipases catalyze the hydrolysis of complex oil molecules, breaking them down into fatty acids and glycerol. Bacteria, such as Alcanivorax borkumensis, utilize lipases to metabolize these breakdown products, converting them into energy and biomass. This bacterial activity aids in the natural degradation of oil spills, promoting ecosystem recovery.
Plastic Degradation	Polyethylene terephthalate (PET) plastic waste	Esterases	PET plastics, commonly used in beverage bottles, are broken down by esterases through hydrolysis of ester linkages. Researchers have discovered bacterial species, such as Ideonella sakaiensis, that produce esterases capable of degrading PET plastics into simpler compounds. This finding opens the door to innovative recycling strategies for plastic waste.
Pesticide Detoxification	Organophosphate pesticides in soil	Esterases	Organophosphate pesticides contain ester bonds that can be targeted by esterases. Soil microorganisms possess esterases that break down these

			pesticides into non-toxic metabolites, reducing their persistence in the environment. This natural detoxification process helps protect soil ecosystems from pesticide accumulation
Wastewater Treatment	Fats, oils, and grease in sewage systems	Lipases	Lipases are employed in wastewater treatment plants to degrade fats, oils, and grease that accumulate in sewage systems. By catalysing the breakdown of complex lipids, lipases prevent pipe clogs and reduce the impact of these pollutants on aquatic ecosystems, ensuring effective wastewater management
Bioremediation of Contaminated Soil	Polycyclic aromatic hydrocarbons (PAHs) in soil.	Various Hydrolases	PAHs, toxic compounds found in soil due to industrial activities, are broken down by a combination of hydrolases. Esterases, lipases, and other hydrolases collaborate to transform PAHs into less harmful compounds that can be utilized by microorganisms or plants. This approach supports the restoration of contaminated sites

6. Applications in environmental restoration:

Hydrolases, with their remarkable ability to catalyze hydrolysis reactions, have found invaluable applications in addressing environmental challenges. From wastewater treatment to soil remediation and contaminated site restoration, these enzymes play a pivotal role in transforming pollutants into harmless compounds [36]. Here are real-world examples of how hydrolases are applied in these contexts:

Environmental restoration	**Application**	**Scenario**	**Hydrolase role**	**Benefits**
Waste Water Treatment	Lipases for Fats, Oils, and Grease (FOG) removal	Municipal wastewater often contains fats, oils, and grease (FOG) from residential and industrial sources. If left	Lipases are introduced into wastewater treatment systems to break down FOG into fatty acids and glycerol through hydrolysis. This	Lipase-based treatment reduces maintenance costs, prevents sewer blockages, and improves the efficiency of wastewater

		untreated, FOG can clog sewage pipes and disrupt wastewater treatment processes	prevents pipe clogs and facilitates the removal of these pollutants during subsequent treatment stages	treatment plants, ensuring compliance with regulatory standards
Soil Remediation	Various Hydrolases for Organic Pollutant Degradation.	Soil contamination by organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs) or pesticides, poses a threat to ecosystems and human health.	A combination of hydrolases, including lipases, esterases, and proteases, collaborate to break down complex organic pollutants into simpler compounds. This sequential enzymatic action occurs in soil microorganisms.	By degrading pollutants, hydrolases contribute to the restoration of contaminated soil, preventing the spread of pollutants and supporting the return of natural ecosystems.
Contaminated Site Restoration	Various hydrolases for Site-Specific Bioremediation	Sites contaminated with a diverse range of pollutants, such as industrial chemicals or oil spills, require site-specific bioremediation strategies.	Tailored enzyme cocktails, including hydrolases like lipases and esterases, are designed to address the specific pollutants present at the site. Microorganisms carrying these enzymes are introduced to the contaminated area, facilitating pollutant breakdown.	Site-specific bioremediation using hydrolases offers a targeted, environmentally friendly solution that minimizes disturbance and promotes the restoration of ecosystems.

7. Examples of successful cleanup endeavors powered by hydrolase-mediated bioremediation:

Hydrolase-mediated bioremediation has proven to be a powerful and sustainable approach for cleaning up various types of pollution [37]. Here are some notable examples of successful cleanup endeavors that have harnessed the enzymatic capabilities of hydrolases:

hydrolase-mediated bioremediation	Pollutant	Hydrolase involvement	Success
Deepwater Horizon Oil Spill Cleanup	Crude oil from the Deepwater Horizon oil spill in the Gulf of Mexicoa	Lipases and other hydrolases produced by naturally occurring marine bacteria	Microorganisms in the ocean naturally produced lipases that played a significant role in breaking down the spilled oil. The microbial community's enzymatic activity contributed to the degradation of the oil over time, aiding in the natural cleanup of the affected areas
Biodegradable Plastic Waste Degradation	Polyethylene terephthalate (PET) plastic waste.	Esterases and other hydrolases capable of breaking down ester bonds in PET plastics.	Researchers discovered Ideonella sakaiensis, a bacterium that produces an esterase capable of breaking down PET plastics. By engineering this enzyme for increased activity, scientists are exploring ways to efficiently degrade PET plastics, reducing plastic pollution.
Soil PAH Contaminant Remediation	Polycyclic aromatic hydrocarbons (PAHs) in contaminated soil	A combination of hydrolases including lipases, esterases, and proteases.	Microbial consortia enriched with specific hydrolases have been used to remediate PAH-contaminated soil. The combined enzymatic action of these hydrolases breaks down the complex PAH molecules into simpler and less toxic compounds, leading to successful soil restoration
Paper and Pulp Mill Effluent Treatment:	Organic compounds in paper and pulp mill effluents.	Various hydrolases, including lipases and cellulases.	Hydrolases have been employed in bioremediation strategies for treating effluents from paper and pulp mills. Lipases break down lipids and fats, while cellulases target cellulose-rich materials present in the effluents, reducing their environmental impact.

Detergent Biodegradation in Wastewater	Surfactants and detergents in wastewater.	Esterases and lipases.	Esterases and lipases are used in wastewater treatment plants to break down surfactants and detergents. These enzymes hydrolyze the ester bonds present in these compounds, rendering them less harmful and facilitating their removal during treatment processes.

8. Engineering nature: Tailoring hydrolases for efficiency

Genetic engineering strategies offer exciting possibilities for enhancing the performance of hydrolases, which are enzymes that catalyze the breakdown of various substrates through hydrolysis reactions [38]. These enzymes have a wide range of applications in industries such as biofuels, pharmaceuticals, and bioremediation. Here are some genetic engineering strategies that can be explored to enhance hydrolase performance.

8.1 Directed evolution: This method involves generating a diverse library of enzyme variants by introducing random mutations in the gene encoding the hydrolase. These variants are then screened or selected for improved activity, substrate specificity, or stability. After multiple rounds of mutation and selection, enzymes with enhanced performance can be obtained.

8.2 Rational design: With a deeper understanding of enzyme structure-function relationships, rational design involves making specific changes to the enzyme's active site or other critical regions. Computational tools like molecular modeling and docking simulations can guide the design of mutations that improve substrate binding, catalysis, or other desired properties.

8.3 Codon optimization: Adapting the gene sequence of the hydrolase to the codon usage preferences of the host organism can lead to increased expression levels and improved enzyme stability. This is particularly relevant when expressing the enzyme in heterologous hosts.

8.4 Chimeric enzymes: By combining different functional domains or active sites from related enzymes, chimeric enzymes can be engineered to exhibit improved performance or new functionalities.

8.5 Site-directed mutagenesis: Specific amino acid residues within the enzyme's active site or other important regions can be targeted for mutagenesis to enhance substrate binding, catalysis, or stability.

8.6 Co-factor engineering: Some hydrolases require co-factors or coenzymes for optimal activity. Modifying the enzyme to accept alternative or non-natural co-factors can expand its substrate range or improve catalytic efficiency.

8.7 Protein engineering libraries: Creating libraries of variant enzymes with diverse mutations can be used for high-throughput screening or selection of improved hydrolase variants.

8.8 Gene fusion and expression optimization: Utilizing bioinformatics tools and structural analysis to identify key regions for engineering can guide the rational design of improved hydrolases.

8.9 Gene fusion and expression optimization: Fusing the hydrolase gene with other protein domains or promoters can lead to enhanced expression, secretion, or stability of the enzyme.

9. Reflection on the potential of hydrolases as eco-friendly biotechnological tools for pollution control:

In a world grappling with the consequences of human activity on the environment, the emergence of hydrolases as potential eco-friendly biotechnological tools for pollution control sparks a sense of optimism. As I ponder over their significance, I am struck by the profound implications that these enzymes hold for shaping a more sustainable future. At the heart of this potential lies the remarkable ability of hydrolases to catalyze the breakdown of diverse pollutants, converting complex, harmful substances into simpler, benign components. [39]. This inherent power to facilitate nature's own restorative processes resonates deeply with the concept of biomimicry – harnessing natural mechanisms for human benefit while minimizing ecological disruption. One of the most compelling aspects of hydrolases is their specificity. These enzymes, honed by evolution, exhibit a remarkable capacity to target particular pollutants with precision. This specificity not only enhances their effectiveness but also mitigates the unintended consequences often associated with traditional pollution control methods. It is an embodiment of the elegance that resides in the subtlety of nature's mechanisms. Equally compel ing is the mild nature of the conditions under which hydrolases operate. Unlike conventional chemical treatments that demand harsh conditions and energy-intensive processes, hydrolases function under ambient temperatures and neutral pH levels. This characteristic not only reduces energy consumption but also aligns beautifully with the principles of green chemistry – minimizing the use of hazardous substances and generating benign byproducts [40]. Considering the potential of hydrolases prompts me to reflect on the concept of circular economy. The ability of these enzymes to transform pollutants into reusable building blocks echoes the principles of recycling and resourcefulness. It's a reminder that solutions to environmental challenges need not be linear and wasteful but can be circular and regenerative. Of course, the journey toward utilizing hydrolases as pollution control tools is not without its challenges. The complexities of certain pollutants and the need for engineering precise enzymatic activities highlight the importance of interdisciplinary collaboration. The translation of laboratory successes to real-world applications also demands the integration of engineering, economics, and policy-making.

10. Overview of ongoing research and novel applications aimed at harnessing hydrolases' full potential

Ongoing research into harnessing the full potential of hydrolases is a testament to their versatility and significance in various fields. Scientists and researchers around the world are exploring novel applications and innovative strategies to leverage these enzymes for a wide range of purposes [41]. Here's an overview of some ongoing research areas and emerging applications:

Sl No	Research areas	Application
1	Bioremediation and Pollution Control	1. Researchers are investigating the use of engineered microorganisms containing hydrolases to target specific pollutants, such as plastic waste, oil spills, and persistent organic pollutants. 2. Enzyme cocktails and microbial consortia are being developed to enhance the breakdown of complex contaminants and improve overall bioremediation efficiency. 3. Studies are focusing on optimizing enzyme production and stability under varying environmental conditions for effective in situ cleanup.
2	Biofuel production	1. Hydrolases play a vital role in converting biomass into biofuels. Ongoing research seeks to improve the efficiency of enzymatic hydrolysis for bioethanol and biodiesel production. 2. Efforts are directed towards engineering enzymes for enhanced cellulose and lignin degradation, crucial for efficient biomass conversion.
3	Textile and Dye Industry	1. Researchers are exploring hydrolases' potential in the textile industry to degrade dyes and remove colour from wastewater. Enzymes can offer a more sustainable alternative to traditional chemical treatments. 2. Novel enzymes with enhanced dye-degrading capabilities are being discovered through metagenomics and protein engineering approaches.
4	Pharmaceutical and Chemical synthesis	1. Enzymatic synthesis using hydrolases is gaining traction as a green alternative to chemical synthesis in the pharmaceutical and fine chemical industries. 2. Researchers are working on expanding the substrate specificity of hydrolases to enable the production of a wider range of valuable compounds.
5	Food and Beverage Industry	Hydrolases are utilized in food processing to improve texture, flavor, and nutritional profiles. Ongoing research aims to optimize enzyme reactions to enhance product quality and reduce waste.
6	Bio-plastics and Polymers	1. Enzymes are being investigated for their role in the production and degradation of bioplastics, offering a more sustainable and biodegradable alternative to conventional plastics. 2. Research is focused on developing enzymes capable of breaking down complex polymers, contributing to a circular economy approach.

7	Personal Care and Household Products	1. Hydrolases are being explored for their applications in formulating eco-friendly detergents, cleaning agents, and personal care products. 2. Studies are ongoing to identify enzymes that can efficiently break down complex ingredients found in these products, reducing their environmental impact.
8	Medical and Therapeutic Applications	Enzymes with hydrolase activity are investigated for various medical applications, including drug delivery systems, diagnostic assays, and therapeutic agents targeting specific diseases.
9	Waste Valorization	Researchers are working on utilizing hydrolases to convert organic waste materials into valuable products, contributing to waste reduction and resource recovery.
10	Nanotechnology	Hydrolases are being integrated into nanomaterials for enhanced enzyme stability, activity, and targeted delivery, opening up new avenues for medical and environmental applications.

Conclusion: Empowering nature's cleanup crew

In the realm of environmental preservation, the role of hydrolases in accelerating pollutant biodegradation stands as a fundamental and transformative force. These enzymatic marvels, evolved by nature over eons, wield a unique ability to catalyze the breakdown of pollutants, breathing life into the prospect of cleaner, more sustainable ecosystems. The elegance of hydrolases lies not only in their potency but also in their adaptability. These enzymes are versatile in their applications, from breaking down complex organic compounds to tackling plastics that have become emblematic of modern environmental challenges. Their actions under mild conditions – often at ambient temperatures and neutral pH levels – signify a departure from energy-intensive, harsh treatments, embracing the principles of sustainability. Perhaps one of the most intriguing aspects of hydrolases is their contribution to the circular economy paradigm. By dismantling pollutants into basic constituents, they offer the promise of transformation, where waste evolves into reusable resources. With the genetic manipulation of microorganisms and the introduction of engineered entities, ethical considerations and robust risk assessments become non-negotiable pillars of progress. In this journey, let us celebrate the beauty of nature's ingenuity, the potency of human innovation, and the harmonious collaboration of both. Let us continue to explore, to innovate, and to advocate for a world where hydrolases continue to be essential catalysts of change in the pursuit of a cleaner, healthier, and more vibrant Earth.

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