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NAGONGERA CAMPUS

DEPARTMENT OF CHEMISTRY

FORMULATION OF GELATIN-CHITOSAN HYDROGEL CONTAINING JATROPHA MULTIFIDA LEAF EXTRACT FOR TREATMENT OF WOUNDS.

BY

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A research project submitted to the Department of chemistry for the partial Fulfillment of the requirements for the Award of the Bachelors of Science Education degree at Busitema university.

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Declaration

I atenia Mathew, declare that the research dissertation is my own original work otherwise cited, and where such has been the case, references have been stated and that the exact study has not been submitted for any award in any other university or tertiary institution of higher education.

Signature .....

Date 13/08/24

Approval

This research review has been submitted for examinationand has been approved by my supervisor.

Dr. Andima Moses.

Signature..... Date.....25/11/2024.

Dedication.

I dedicate this report to my parents and my supervisor. Mr Okiria moses , Akwii Judith Oliver and Dr Andima Moses my supervisor, my siblings Apio Marion, Orena Paul, Tino Esther and Ogwang David. And my lovly grandmother Anyait Rose not forgetting my uncles, that is okalany David livingstone, Ocom Samuel and Orena Joseph who have supported me in all aspects of my entire life journey of pursing my career dream as a professional teacher.

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Abstract.

When the wound arises, there are some effects that will arise as the loss of all or part of the organ functio, bacterial contamination. Wound healing healing can be with the use of traditional medicine. Some traditional remedies such as iodine plant (jatropha multifida) have been used to accelerate wound healing maximumly. Due to emergency of resistant bacterial strains and significant increase in side effects of currently available antibacterial drugs have made it urgent to develop research to identify new bioactives antibacterial compounds and innovative ways for drug delivery to enhance wound healing. This research aims to developa novel hydrogel formulation that combines the beneficial properties of gelatin, chitosan and jatropha multifida leaf extractfor effective wound healing.

Hydrogel composed of gelatin, chitosan, and jatropha leaf extract can be one of the right candidates for wound dressing application which provides both an antibacterial and a proper wound drainage management property to promote faster healing. Herein, preparation of hydrogel has been conducted by the physical blending of the solution of gelatin, chitosan, and extract at $4O^0C$. Then, the mixture was cast to form hydrogel films by each 2-4 mm thickness and followed by drying at 37^o C for 24 hours. The resulted hydrogels were characterized to confirm its potential as wound care dressing by gel fraction, swelling index, and antibacteria. The gel fraction of the hydrogel composed of 10 and 20 grams of gelatin (each with 0.5 grams of chitosan and 5ml grams of the extract) was respectively 68.86 % and 65.68%. The hydrogel, composed of 20 g of gelatin and 7.5 g of chitosan, has shown the highest water retention capacity (swelling index) by 400 %. However, the presence of the extract has slightly lowered both the gel fraction and swelling index of the resulted hydrogel. Jatropha multifida, a plant with known antimicrobial and anti-inflammatory properties, is being explored for its potential application in wound care. The combination of these components in a hydrogel formulationhas the potential to create a synergistic effect that promotes wound healing by providing a moist environment, antimicrobial activity,and anti-inflammatory effects.

The research involved extraction of the plant bioactive components, phytochemical analysis, antimicrobial investigation of the leaf extract of the plant and formulation of the hydrogel.

Methods. The extract was obtained by maceration, phytochemical screening of jatropha multifida was carried out. The antimicrobial activity of of the leaves was evaluated on the strains of s. aureus, and E.coli**. Results**: the presence of flavonoids, tannins, alkaloids, saponins was noted on the leaves of jatropha multifida. The ethanolic extract of the leaves did not show effective bacterial inhibition**. Conclusion,** this study holds promise for the development of an advanced wound dressing material that harnesses the therapeutic potential of natural ingredients combined with biocompatible polymers. The successful formulationof gelatin- chitosan based hydrogel loaded with j. Multifida leaf extract could offer a cost effective and sustainable approach to wound managementwith reducing reliance on synthetic materials and promoting the use of natural resources for medical applications.

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CHAPTER ONE: INTRODUCTION.

1.1 Background of the study.

Damage to a portion of the body's tissues where there is specific tissue substance that is damaged or lost is a wound condition(Stroncek & Reichert, 2008) . When wounds arise either acute or chronic wounds there are several effects that will arise such as loss of all or part of organ function, sympathetic stress response, bleeding,pain and blood clots, bacterial contamination and cell death(Hatz, Niedner, Vanscheidt, & Westerhof, 2012).

Acute wounds are injuries that occur in tissue that can recover in a minimum of 8-12 weeks. The main cause of acute injury is mechanical injury due to external factors, which occur as a result of contact between the skin with a hard or sharp surface, open wounds, and post-operative injuries. Other causes of acute injuries are burns and chemical injuries, such as exposure to radiation rays, electric shock, exposed to corrosive chemicals.

When there is no further response to the wound,the impact is irritation, infection, inflammation, fever and the worst effect is tetanus(Iqbal & Shahid, 2004).Untreated wounds can also result into impairementof normal functioningof the affected body parts which can either hinder locomotion, causes pain or lead to partial or complete loss of function.

Wound healing is a natural process by which the body repairs damaged tissue(Reinke & Sorg, 2012). The wound healing process is not only limited to local regeneration process, but is also influenced by endogenous factors, such as, nutrition immunology, drug use, and metabolic conditions(Eming, Hammerschmidt, Krieg, & Roers, 2009). It involves several stages including hemostatis,inflammation,proliferation and remodeling(Landén, Li, & Ståhle, 2016).

wound therapies include primary intention healing,secondary intention healing,tertiary intention healing,topicaltherapies,negativepressurewoundtherapy,biological therapies,debridement,hyperbaric oxygen therapy,vacuum assisted wound closure and many others(Kesl, 2016).

Wound heaing is a complex and dynamic process that involes various stages mentioned above.

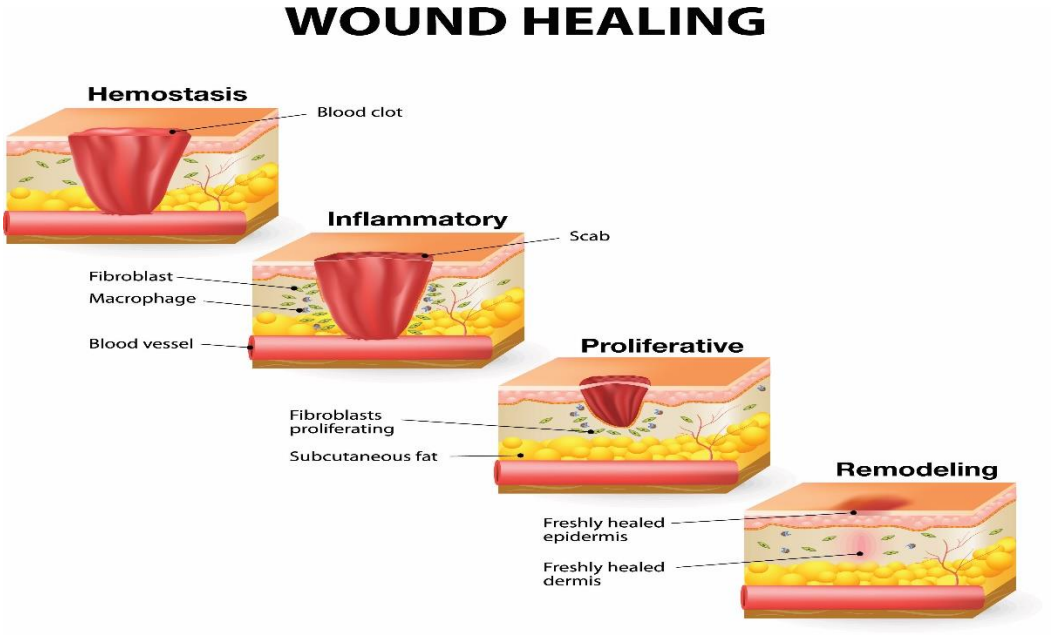

Figure 1 shows Wound healing processes

In recent years,natural polymers have gained significant attention as potential wound dressing materials and drug deli very systems due to their biocompatibility, biodegradability and ability to promote cell adhension and proliferation(Yaşayan, Alarçin, Bal-Öztürk, & Avci-Adali, 2022) Gelatin-chitosan hydrogel is a biocompatible and biodegradable polymeric system that has been widely investigated for various biomedical applications including tissue engineering and drug delivery.The combination of gelatin and chitosan results in a hydrogel with improved mechanical properties ,swelling capacity and mucoadhensive properties.(Ebhodaghe, 2022)

In recent years, it has been quite popular among rural population to use herbs and their products as medicine to treat wounds. The world health organization estimated that about 80% of the world's inhabitants use traditional medicine as primary health care(Organization, 2009). The increased use of medicinal plants was explained by their availability and the accessibility in developing countries like uganda and that herbs contain many beneficial pharmacological active compunds and are widely accepted that such compounds are more tolerable and cheaper than synthetic chemicals and that they can be processed into safe drugs . One of the traditional plants widely used by people is jatropha multifida .The aim of the present study was to formulate chitosan-gelatin hydrogel containing J. multifida leaf extract for wound healing(Morguette et al., 2023).

1.2 Problem statement

Chronic wounds present a significant healthcare challenge worldwide ,often leading to prolonged hospital stays ,increased healthcare costs and decreased quality life of patients(Sen, 2019). About six million people in the world are suffering from chronic wounds, and the government suffers the cost of seven billion dollars at the global level for long-term treatment of wounds(Organization & Canada, 2005) . Since economic and social costs of existing wound treatment are high for patients and goverments and diverse side effects of antibiotics such as hypersensitivity, allergic reactions and immunosuppression, in addition the increasing prevalence of multidrug-resistantstrains of pathogenic microorganisms constitutes an important and growing threat to public health. Therefore it is necessary to find new medicines which can be applied as alternative or complementary therapies. Natural products ,such as plant extracts have gained attention for their potential therapeutic effects in wound healing due to their diverse biochemical composition and minimal side effects. Jatropha multifida, a plant rich in bioactive compounds such as flavonoids, phenolics and alkaloids has shown promising wound healing properties in traditional medicine(Sabandar, Ahmat, Jaafar, & Sahidin, 2013).

However, the direct application of the plant extracts to wounds maybe limited by their low solubility and stability as well as potential toxicity concerns at higher concentrations. Therefore the development of a suitable delivery system that provide sustained release of bioactive compounds while promoting wound healing.

Hydrogels composed of biocompatible polymers such as gelatin and chitosan have emerged as promising candidates for wound dressings and drug delivery medium due to their biocompatibility,biodegradability and ability to absorb wound exudates(Jacob et al., 2021). This research aims at addressing the need for an effective wound healing formulation by developing a gelatin –chitosan hydrogel loaded with jatropha multifida leaf exract.

1.3 Research questions

1 What is the optimal ratio of gelatin to chitosanin the hydrogel formulation containing jatropha multifida leaf extract for effective wound healing.

2 How does the concentration j.multifida leaf extract affect the antimicrobial propertiesof gelatin-chitosan hydrogel for wound healing

1.4 Objectives of the study

1.4.1 General objectives

To formulate gelatin-chitosan hydrogel conyaining jatropha multifida leaf extract for wound healing.

1.4.2 Specific objectives.

To extract the compontnts responsible for wound healing in j. multifida plant leaves.

To qualitatively determine some of the phytochemicals present in j. multifida plant leaves.

To investigate the antibacterial activity of j. multifida leaf extract.

To synthesize and classify hydrogels

1.5 Scope of the study

1.51 Geographical scope

The research covered tororo district in eastern Uganda. The study area was covering selected s ub counties in tororo district that is kayoro and osukuru sub county in Tororo south county inTororo district.

1.5.2 Content scope.

The study was focused on introduction,literature review ,materials and methods , results and discussion.

1.5.3Time scope

The research was carried out for a period of three months ,commencing from February to april as specified according to the semester basis.

1.6 Signifance of the study.

The information obtained from this study of formulation of gelatin-chitosan hydrogel loaded with jatropha multifida leaf extract for wound healing can be used by the local community, government or authorities and ngos like who to sensitize and create awareness for development of evidence based wound care strategies utilizing natural products such as plant extracts as natural remedy for affordable, available and quick wound healing remedies.

The study also act as a footstep for researchers to establish effectiveness of more local processing methods of obtaining jatropha multifida leaf extract.

The study provides baseline information and knowledge to both the government and local population the importance of herbs in treating various diseases particularly wounds as cheap, local and affordable remedy.

1.7Justification of the study.

There are health threats associated with wounds as result of leaving them untreated. These health effects include rotting of the affected part, unpleasant smell, head ache , fatigue, tetanus disease which can cause mental disorders, can also deform the affected body part and death for several cases in Tororo district. jatropha multifida is commonly knowns as coral plant or physic nut and it is widely distributed in the tropical regions throughout the world. It is a multipurpose medicinl agent but there was little usage as a wound healing agent so there was need to raise awareness on use of jatropha multifida leaf extact as remedy for wounds and the study provided a baseline information and there was need to assess the effectiveness of jatropha multifida leaf extract in wound healing proces

CHAPTER TWO : LITERATURE REVIEW.

A wound is damage of tissue or organs accompanied by distraction of the skin structure or mucous membrane(Sorg, Tilkorn, Hager, Hauser, & Mirastschijski, 2017), which plays an important role in preventing water loss and blocking the invasion of harmful substances and pathogenic microorganisms as significant interferace between the body and it's surrounding.

Wound healing is body's natural reaction to tissue injury involving a series of cellular events that generates resurfacing, reconstitution, and restoration of the tensile strength of injured skin. Wound healing is a complex and dynamic process which involves blood coagulation, formation of fibrin network re-epithelialisation, collagen maturation and remodeling of connective tissue(Cowin & Waters, 2014). The main aim of carrying out treatment is to obtain complete regeneration and restoration of the skin structure without altering the skin's function with minimal or no scarring(Metcalfe & Ferguson, 2007). Tissue repair is a linear process where certain growth factors are involved which cause proliferation of the cell which lead to certain dynamic changes that involve blood cell,soluble mediators, production of extracellular matrix and proliferation of extracellular matrix(Raghow, 1994). Sometimes tissue repair failure can occur because of malfunctional skin responses.

Wound care is constantly evolving with advances in medicine but wounds are still a significant health problem worldwide, often having severe complications(Natarajan, Williamson, Stiltz, & Harding, 2000).

In recent years wound care professionals revisited the ancient healing method by using traditional medicine in wound management. These traditional medicines are more effective, non toxic and cost effective and they have been recognized as a rich source for the development of pharmaceuticals, this has made them to gain popularity among people worldwide.

The genus jatropha belongs to the family Euphorbiaceae and has great variety of species, among them is j. multifida,j. curcas, j. molissima, j. gossypifolia that are currently the source of studies for the production of biodiesel and also medicinal character(Devappa, Makkar, & Becker, 2010a).

Jatropha multifida commonly called coral bush, is characterized as a shrub up to 1.30m high, and can reach 7m in its habitat. It has green leaves up to 17cm long and 23cm wide, red inflorescence up to 20cm long, fruit upto 3cm long and 2.5 cm in diameter.

Figure 2 Jatropha multifida plant

The entire plant has whitish latex, so the leaves, stem, bark and roots of jatropha plant have been used in ethno- medicines. Jatropha multifida is used in African folk medicine for the treatment of infection, pain,fever, various inflammatory conditions, tumor and tumor related diseases(Falodun, Igbe, Erharuyi, & Agbanyim, 2013).

The genus jatropha is a rich source of phytochemicals that can be utilized in nutritional, agricultural, and pharmaceutical industries(Devappa, Makkar, & Becker, 2010b). The pharmaceutical properties presented by the plant are diverse,being the latex produced by it the substance that presented the highest therapeutic potential described in the literature to date.

The plant is known to possess different medicinal properties including antimicrobial activity,anti-inflammatory and antioxidant activities,phytochemical hemolytic, toxic and larvicidal properties. The stems of j. multifida could be regarded as an anti-influenza herbal medicine as well as a potential crude drug source for the developmrnet of anti-influenza compounds(Vieira et al., 2021). The leaves,the latex and the fruits of this plant are used in the treatment of wounds, skin infection and as a cicatrizing wounds,ulcers,oral thrush, constipation and fever. Roots and stems are used as anticancer, antitumor, cytotxic, antimalarial, amtimicrobial, insecticidal an d molluscidal because

of the presence of diterpenoids lathyrane. Its bark and leaves are used as medicine for neurodermatitis, ichy skin and skin eczema.

2.1 Hydrogels and wound healing.

Hydrogel is a water-swollen, and cross-linked polymeric network formed by the simple reactionof one or monomers(Malpure, Patil, More, & Nikam, 2018). Another definition is that it is a polymeric material that exhibits the ability to swell and retain a significant fraction of water within its structure, but will not dissolve in water(Po, 1994).

Hydrogels are soft moist or jelly-like materials as a result of their three-dimensional network developed by many cross-linked polymers which enable to retain a high quantity of adsorbed water. Hydrogel wound dressing is composed of natural and synthetic polymers, which can absorb tissue fluid, improve the local microenviroment of the wound, and promote wound healing(Khandan, Jazayeri, Fahmy, & Razavi, 2017).

polymers are cross-linked through their functional groups either by chemical bonds or physical interactions such as hydrogen bond, ionic interaction, and hydrophobic forces. Both synthetic polymers and natural polymers have been used to develop various hydrogels. Many hydrogels show their unique features in response to both chemical and physical stimuli(Bashir et al., 2020). Depending on its polymer structure, hydrogels also can resemble an extracellular matrix. These outstanding chemical, physical, and biological properties make their extensive applications as biomedical materials, for instance, tissue engineering, drug delivery system, and wound care dressing(Ho et al., 2022).

The holding capacity and permeability are the most characteristic features of the hydrogel. The polar hydrophilic groups are the first to be hydrated upon contact with water which leads to the formation of primary bound water(Karoyo & Wilson, 2021). As a result the network swells and exposes the hydrophobic groups which are also capable of interacting with water molecules. This leads to formation of hydrophobically-bound water also called secondary water.

Primary and secondary bound water are often combined and called total bound water'. The network will absorb the additional water, due to osmotic driving force of the network chains towards infinite. The ability of hydrogels to absorb water arises from hydrophilic functional groups attached to the backbone, while their resistance to dissolution arises from cross-links between network chains(Lu et al., 2018).

Hydrogel-based materials have been used as primary dressing materials,especially for managing moist wounds(Divyashri et al., 2022). Hydrogel-based materials absorb a high amount of wound moisture to form a viscous gel that allows the excess fluid removal. In another way, hydrogels are also capable of keeping the humid environment around the wound that is important to enhance re-ephitelialisation. Their The soft and non-adhesive properties give an advantage by preventing wound damage during their removal.Three- dimensional network structure of hydrogel can facilitate tissue growth, macrophages activation, interleukin production to stimulate the cell adhesion and proliferation to complete wound healing process(Norahan, Pedroza-González, Sánchez-Salazar, Álvarez, & de Santiago, 2023).

2.1.1 Properties of hydrogels.

Hydrophilic gels called hydrogels receive considerable attention for their use in the field of pharmaceutical and biomedical engineering(Kashyap, Kumar, & Kumar, 2005).

2.1.1.1 Swelling properties. A small change in environmental condition may trigger fast and reversible changes in hydrogels. The altermation in environmental parameters like electric signal, ph, temperature, and presemce of enzymw or other ionic species may lead to a change in physical texture of the hydrogel(Gholamali, 2021).

2.1.1.2 Mechanical properties. The desired mechanical property of the hydrogel could be achieved by changing the degree of of cross-linking and increasing the degree of cross-linking. A stronger hydrogel could be achieved through the higher degree of cross-linking decreases the percentage elongationof the hydrogels creates a more brittle structure(Hu, Liang, Li, Liu, & Sun, 2019).

2.1.1.3 Biocampatible property. Is the abilityof the material to perform within an appropriate host response in specific applications. It basically consists of two elemnets that is biofunctionality which is the ability of the material to perform specific task which it was intended for and the second one is biosafety is to appreciate host response not only systematic but also local, the absence of mutagenesis, cytotoxicity, and carcinogenesis.

2.1.1.4 High water absorption and retention.

Hydrogels have cross-linked networks,so they can dissolve and retain a large amount of water, and the water content can be as high as 99%. The dressing made of hydrogel absorbs the wound exudate while maintaining a most environment, so it will cause secondary trauma by adhension with the wound(Zhang, Liu, Zhang, & Pei, 2020). And the hydrogel absorbs a large a mount of

fluid, so it does not need to be changed frequently. In addition, the surface of the hydrogel is smooth and highly elastic, and when used as a dressing, it can fit closely to thr wound without adhension and reduce bacterial contact, which is the most advanced medical dressing at present(Bibi, 2011).

Advantages of hydrogels made from gelatin-chitosan loaded with jatropha multifida leaf extract.

They possess a degree of flexibility very similar to natural tissue due to their significant water content.

They are biocompatible, biodegradable and canbe injected. Hydrogels also possess good transport properties and easy to modify. Environmentally sensitive hydrogels have the ability to sense changes of pH, temperature, or the concentrationof metabolite and release their constitutes as a result of such changes.

chitosan has a potential hemostatic feature which helps natural blood clotting and block nerve ending to reduce pain(Fan, Zeng, Zaldivar-Silva, Agüero, & Wang, 2023). Gelatin has an excellent filmogenic property, which allow the production of film. Besides, its gelation property can act as a binding agent that constricts the vessel and stop the blood flow. The chitosan and gelatin blending is usually conducted to improve the mechanical strength as well as to inhibit rapid biodegradation of the hydrogel(Ebhodaghe, 2022).

Jaropha multifida leaf extract a traditional wound care material, can be a right candidate as a bioactive agent in the gelatin-chitosan hydrogel to improve the healing effectiveness. This work aimed to prepare chitosan gelatin hydrogel loaded with j. multifida leaf extract for enhanced wound healing.

2.2 Preparation of hydrogels.

Hydrogels can be prepared from either synthetic polymers or natural polymers. The synthetic polymers are hydrophobic in nature and chemically stronger compared to natural polymers. Their mechanical strength results in slow degradation rate, but on the other hand, mechanical strength provides durability as well. Possibly also by reacting polymers with suitable cross-linking agents.

Natural compounds	Synthetic compounds
Gelatin	Polyethylene glycol (PEG)
Chitosan	Polycaprolactone (PCL)
Fibrin	Polyvinylpyrrolidone (PVP)
Hyaluronic acid	Poly(l-Lactic acid) (PLA)
Alginate	Poly(lactic-co-glycol)

Table 1 synthetic and natural compounds most commonly used in preparation of hydrogels

2.3 Hydrogel classification.

Hydrogels have physical properties similar to living tissue due to their high water content, softness, and plasticity. They can be classified in several ways depending on the parameter that will be taken as a differentiating factor(S. C. Lee, Gillispie, Prim, & Lee, 2020). Hydrogels can vary due to the source of origin, ionic charge, size, crosslinking methods, chain composition, biodegradability, and response to different factors.Characterizations of each class of hydrogels are detailed. However, each type of hydrogel has different physical, chemical, and biological properties due to which they can be used for a wide variety of application

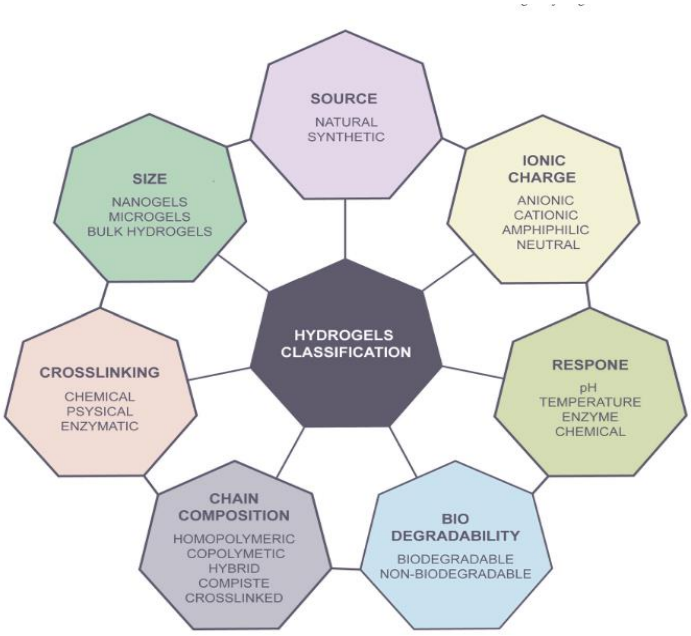

Figure 3 classification of hydrogels

2.3.1 Source.

Due to the source of origin hydrogels are divided into natural andsynthetic polymers. Natural polymers are biomolecules found in nature that are made up of repeating subunits(Dey, Mitra, & Alam, 2023). They are formed through biological processes and can be found in many living organisms, such as plants, animals, and microorganisms. The group of hydrogels with natural origin mainly includes collagen, gelatin, fibrin, hyaluronic acid, Matrigel, alginate, and others. On the other hand, synthetic polymers are man-made polymers that are produced through chemical reactions in a laboratory. While both natural and synthetic polymers have their unique properties and applications, they share some common characteristics, such as high molecular weight, flexibility, and the ability to be formed into various shapes and sizes(Asti & Gioglio, 2014)

2.3.1.1. Natural polymers.

The natural hydrogels most used as biomaterials are described below.

2.3.1.1.1. Gelatin.

Gelatin is made of the same amino acids as collagen. Gelatin is derived from collagen through a process of hydrolysis, which breaks down the collagen molecules into smaller peptides(Alipal et al., 2021). It is obtained by partial acid hydrolysis (type A gelatin) or by partial alkaline hydrolysis (type B gelatin) of collagen from the animal bones, skin, and muscles. This process involves heating collagen in water, which causes the collagen fibers to unravel and release the smaller peptides. These peptides can then be further processed to form gelatin. Besides being widely used

in the food industry, gelatin also has several beneficial biological properties that make it ideal for use in engineering and regenerative medicine applications. Its biocompatibility, low antigenicity, biodegradability, and ability to stimulate cell growth and attachment make it an invaluable resource for these fields. Gelatin is widely used as a biomaterial in various medical applications. It serves as a tissue engineering scaffold, providing support for cell growth and tissue regeneration(Nikkhah et al., 2016). Gelatin-based dressings promote wound healing by creating a favorable environment. It is also utilized for controlled drug delivery and as a hemostatic agent during surgical procedures(Ndlovu, Ngece, Alven, & Aderibigbe, 2021)

2.3.1.1.2 Hyaluronic acid (HA).

Hyaluronic acid (HA) is a polysaccharide belonging to the group of glycosaminoglycans. Its structure consists of alternating mers of D-glucuronic acid and N-acetyl-Dglucosamine linked by β(1→4) and β(1→3) glycosidic bonds(Iordache, Mihai Grumezescu, Maniu, & Curutiu, 2017). HA is well known for its strong hydrophilicity due to the numerous carboxyl groups that can form hydrogen bonds with water molecules. This property enables 1 g of HA to bind up to 6 liters of water, making it an ideal component for numerous cosmetic products. Such products are favored due to the biocompatibility, biodegradability, low toxicity, viscosity, and plasticity of HA. HA is commonly used in tissue engineering as a scaffold for promoting cell adhesion, proliferation, and tissue regeneration(Hemshekhar et al., 2016). HA-based dermal fillers are used for cosmetic purposes to reduce the appearance of wrinkles and restore volume to the skin. It is utilized in ophthalmic surgeries as a viscoelastic agent to protect and lubricate the eye. Additionally, HA plays a crucial role in drug delivery systems, facilitating controlled release of pharmaceuticals(Liao, Jones, Forbes, Martin, & Brown, 2005).

2.3.1.1.3 Fibrin.

Fibrin belongs to the group of fibrillar proteins, formed from fibrinogen through the activity of thrombin. Fibrin has natural biostatic and bioactive properties(Mogford, Tawil, & Mustoe, 2004). It induces processes such as angiogenesis, synthesis of cytokines and ECM, and enhanced cell migration and proliferation. Fibrin plays a very important role in the aspect of regeneration, i.e. filling tissue defects. The mechanical strength of fibrin gel can be regulated by increasing or decreasing the presence of divalent ions such as Ca2+. Fibrin hydrogels have several advantages as a biomaterial, including their biocompatibility, biodegradability, and ability to support cell growth and differentiation. Fibrin, as a biomaterial, has many applications in medicine. It serves as a scaffold for tissue engineering, facilitating cell growth and regeneration. Fibrin-based

hemostatic agents effectively control bleeding during surgeries. It is utilized as a drug delivery system, enabling controlled release of therapeutic agent.

2.3.1.1.4 Alginate.

Alginate is a well-known polysaccharide extracted from marine algae, especially from brown algae (Phaeophyceae). It's a native co-polymer consisting of β -D-mannuronic acid (M-blocks) and α -L-guluronic acid (G-blocks) residues linked by glycosidic bonds. The M and G blocks can occur in different proportions and different arrangements along the polymer chain. Alginate possesses strong sorption properties, with low total pore volume and internal surface area. Its high water-binding capacity is achieved through hydrogen bonding between the -OH groups on alginic acid fibers, as well as to unsubstituted groups in the case of divalent metal alginates. The process of alginate hydrogel formation is triggered by divalent ions, especially Ca2 +. Alginate hydrogels are used in drug delivery systems, allowing controlled release of pharmaceuticals. Alginate-based dressings are used for wound healing, providing a moist environment to promote tissue regeneration

2.3.1.2 Synthetic polymers.

The second group of hydrogels consists of synthetic-derived hydrogels. Synthetic-derived hydrogels are produced through a controlled process in which monomers are polymerized to form macromolecules with distinct properties. This process can be used to create synthetic polymers with specific physical and chemical characteristics that enable them to be used for a wide variety of applications. The synthetic polymers most used as biomaterials are described below.

2.3.1.2.1 Polyacrylamide.

Polyacrylamide (PA), a water-soluble linear polymer, is composed of acrylamide monomers or a combination of acrylamide and acrylic acid monomers. This polymer is widely used in agriculture, food processing, and other industries. Its hydrogel form consists of a covalent polymeric network combined with varying amounts of water. The water content in polyacrylamide hydrogel can range from 70% to 90%, making it extremely useful for applications such as absorption, filtration, sedimentation, and flocculation. PA hydrogels have several advantageous properties that make them suitable for a range of biomedical applications. Due to their composition and extensive crosslinking, these materials are highly biocompationenabling safe and non-toxic interactions with living cells. Furthermore, the physical and chemical properties of PA hydrogels can be tailored by adjusting their composition or adding functional groups or bioactive molecules. This allows for tuning the mechanical properties to match those of healthy tissues and organs in the human body.

Moreover, such hydrogel scaffolds are capable of creating 3D structures that closely resemble the ECM, further increasing their potential use in TE strategies

2.3.1.2.2 Polyethylene glycol.

Polyethylene glycol (PEG) is a polyether compound derived from petroleum, consisting of long chains of ethylene oxide molecules. It exhibits excellent water solubility and has been used extensively in biomedical applications due to its biocompatibility and ability to be modified covalently with functional groups and bioactive molecules, allowing for tuning of mechanical properties of hydrogels to match those found in tissues within the body. PEG-based hydrogels are popular in medical applications as they possess many advantageous characteristics. For example, these hydrogels can be formed into a variety of shapes and sizes, enabling them to fit into specific anatomic locations. Furthermore, PEG-based hydrogels are capable of controlling drug release kinetics by altering the mesh size or incorporating chemical moieties that can respond to external stimuli such as temperature or pH levels.

2.3.1.2.3 Peptide hydrogels.

Peptide hydrogels are hydrogels composed of short peptide chains that self-assemble in water to form a 3D network structure. Peptides are short chains of amino acids and can be designed to have specific chemical and physical properties. By controlling the sequence and concentration of the peptides, it is possible to create hydrogels with a wide range of properties, such as stiffness, porosity, and biocompatibility. Peptide hydrogels are attractive materials for tissue engineering and regenerative medicine applications because they can mimic the ECM of natural tissues. The ECM is a complex network of proteins and other molecules that provides structural support and biochemical signals to cells.

2.3.1.2.4 Hybrid hydrogels.

Hybrid hydrogels are a rapidly expanding class of materials that show promise for biomedical applications due to their versatility, functionality, and improved performance over pure polymer networks. To create hybrid hydrogels, two or more different types of polymers must be combined, such as natural polymers (e.g., gelatin, chitosan) and synthetic polymers (e.g., polyethylene glycol). These combinations of polymers can provide increased mechanical strength and biocompatibility compared to purely synthetic hydrogels. Furthermore, these hybrid hydrogels can have their properties tailored by different concentrations and types of polymers, as well as by controlling the degree of crosslinking between them. This ability to tailor properties enables hybrid

hydrogels to be used in a wide range of applications, including tissue engineering, drug delivery, and diagnostic biosensors.

2.3.2 Size of particles.

Hydrogels can be classified based on various characteristics, including their chemical composition, crosslinking density, and mechanical properties. Another way to classify hydrogels is based on the size of their particles(Garg, Garg, & Vishwavidyalaya, 2016). Based on particle size, three types of hydrogels can be identified: nanogels, microgels, and bulk hydrogels. The size of the particles in a hydrogel can have a significant impact on its properties and potential applications(Daly, Riley, Segura, & Burdick, 2020). By selecting the appropriate size and characteristics of the particles, hydrogels can be tailored to meet specific requirements for a wide range of biomedical and biotechnological applications. Particles smaller than 100 nm form nanogels, subsequently microgels are formed by particles reaching micrometric size. Hydrogels with molecules larger than 100 μm are called bulk hydrogels Microgels, due to the high presence of solvent in their structure, usually form soft structures(Farjami & Madadlou, 2017). According to that, they are prone to exchange solvents with the fluid that is in the external environment of the hydrogel. Microgels, due to their hydrophilic nature, have a high ability to absorb water, and their swelling properties are reversible depending on external stimuli, which can be a change in temperature, pH, ionic strength, and solvent. Nanogels have high water absorption and swelling. This is caused by the presence of hydrophilic functional groups such as -OH, -CONH-, -CONH2-, -COOH, and -SO3H(Atta, Al-Lohedan, ALOthman, Abdel-Khalek, & Tawfeek, 2015). Due to the hydrophilic nature of the nanogels, they have a wide range of properties such as biocompatibility and high transferability of hydrophilic biotherapeutics, high stability, and biodegradability

2.3.3 Crosslinking.

Crosslinking is a critical step in the formation of hydrogels, as it imparts structural stability, mechanical strength, and biocompatibility to the material. Chemical, physical, and enzymatic crosslinking can be distinguished(Bashir et al., 2020). Hydrogels need to be cross-linked to maintain their shape and integrity, as well as to control their properties and functionality. Crosslinking refers to the process of linking polymer chains together to form a 3D network structure, which gives hydrogels their unique properties, such as high water content, softness, and flexibility(J. Li, Jia, & Yin, 2021). Crosslinking is an essential step in the formation of hydrogels, as it provides the structural stability, mechanical properties, and biocompatibility needed for a

wide range of biomedical and biotechnological applications(Saroia et al., 2018). The enzymatic method is one of the most gentle ways of crosslinking. Enzymes mostly exhibit a high degree of substrate specificity. This allows for the avoidance of potential side reactions during the process and makes it possible to control and predict kinetics, and the overall crosslinking rate(Punekar, 2018). An example of enzymatic crosslinking of hydrogels is the crosslinking of gelatin and chitosan in the presence of transglutaminase and tyrosinase. Chemical crosslinking of hydrogels involves the use of covalent bonding between polymer chains to produce a stable hydrogel. Most commonly, small crosslinking molecules, polymer-polymer conjugation, or photosensitizers are used for this purpose. Covalent bonds are formed primarily between functional groups of polymers (-OH, -COOH, -NH2), which provide solubility to water-soluble polymers(Akelah & Akelah, 2013). Covalent bonds are formed between two groups that have complementary reactivity. Among the most commonly used reactions are Schiff base formation, Michael addition, peptide bonds formation, and "click chemistry'' reactions . Physical crosslinking of hydrogels refers to the formation of a 3D network structure through physical interactions between polymer chains, rather than through chemical reactions(Akelah & Akelah, 2013). This process typically involves the use of non-covalent interactions, such as hydrogen bonding, hydrophobic interactions, or electrostatic interactions, to create a stable network. There are several ways to physically crosslink hydrogels, including temperature-induced gelation, ionic gelation, and hydrogen bonding. Physical crosslinking has several advantages over chemical crosslinking, including better biocompatibility, easier fabrication, and the ability to reversibly change the properties of the hydrogel. However, physical crosslinking can also be weaker than chemical crosslinking, and the properties of the hydrogel may be more sensitive to changes in the environment, such as temperature or pH (Maitra & Shukla, 2014).

2.3.4. Biodegradability.

The biodegradability of hydrogels is a highly d esirable trait for numerous biomedical applications, particularly those that require the use of these materials as temporary scaffolds for tissue regeneration or drug delivery(Song et al., 2018). Depending on the intended purpose, hydrogels can be designed to be biodegradable or non-biodegradable, depending on the intended use and application. Biodegradable hydrogels can break down into smaller molecules over time, which allows for their safe elimination from the body or environment(Sannino, Demitri, & Madaghiele, 2009). There are several methods for achieving biodegradability in hydrogels, including using natural or synthetic polymers that are inherently biodegradable,

incorporating cleavable chemical bonds into the hydrogel structure, or designing the hydrogel to respond to specific environmental cues that trigger degradation(Kharkar, Kiick, & Kloxin, 2013). In the context of biomedical applications, biodegradable hydrogels are particularly attractive for drug delivery, tissue engineering, and wound healing. They can be designed to release therapeutic agents over time while gradually breaking down and being eliminated from the body. It is very important to be able to control the mechanism and rate of degradation, for example, too fast degradation of the scaffold may not be appropriate for the cells to produce enough ECM, and slow degradation may affect cell migration and other processes essential for cell survival. Biodegradability can also be harnessed for controlled drug release, e.g. peptides specific to enzyme degradation. For example, Zisch et al. prepared degradable hydrogel with the use of MMPs degradable peptides crosslinked within the PEG matrix functionalized with a vinyl sulfone. Non-biodegradable hydrogels, on the other hand, are designed to be long-lasting and remain in the body for extended periods. They are typically used in applications where a long-term, durable support structure is needed, such as in contact lenses, wound dressings, or artificial implants. Non-biodegradable hydrogels are not designed to degrade, so they can potentially cause long-term harm to the body if they are not removed or replaced over time

2.3.5. Ionic charge.

Ionic hydrogels, or polyelectrolytes, are a type of hydrogel composed of monomers with specific ionic charges(C.-J. Lee et al., 2018). These can be divided into four distinct categories based on the charge: anionic hydrogels, which contain negatively charged ions; cationic hydrogels, which contain positively charged ions; amphiphilic hydrogels, which contain both positively and negatively charged ions; and neutral hydrogels, which have no net charge(Mocanu & Nichifor, 2014). Anionic hydrogels include alginic acid, pectin, HA, carrageenan, dextran sulfate, chondroitin sulfate chondroitin(Vasiliu, Racovita, Popa, Ochiuz, & Peptu, 2019). Anionic hydrogels show a definite increase in swelling coefficient as the pH of the environment increases. Anionic hydrogel networks are usually defined as homopolymers of negatively charged acidic or anionic monomers or copolymers of an anionic monomer and a neutral monomer. Chitosan and polylysine, which have a positive charge are examples of cationic hydrogels. Cationic polymeric networks could also be derived through modifications such as partial hydrolysis of the existing non-ionic pre-formed polymer networks(Tang et al., 2016). Cationic pendant groups in a polymer network on the contrary behavior to anionic pendants give rise to hydrogels, which remain collapsed in the basic environment and swollen in the acidic environment due to the electrostatic

repulsion between the positively charged groups. Polyampholytic hydrogel networks are referred to as macromolecules capable of possessing both positively and negatively charged moieties in the polymer network(Kudaibergenov, 2002). Subsequently, the group of amphiphilic hydrogels can include, for example, collagen, gelatin, carboxymethylated chitin, fibrin, and starch. The last group of natural hydrogels, which includes such polymers as dextran, agarose, and pullulan, is an example of neutral hydrogels(Wasupalli & Verma, 2018).

2.3.6 Response to stimuli.

Hydrogels can be further categorized according to their reaction to external environmental stimuli, such as pH, temperature, or enzyme(Bustamante-Torres et al., 2021). Responses may range from a physical change in the material's shape or structural integrity to changes in permeability, solubility, or optical properties. The first group of hydrogels may include pH-responsive hydrogels. They change their volume in response to changes in the pH of the surrounding environment. This volume change can be due to changes in the ionization state of acidic or basic functional groups in the hydrogel polymer. Examples of pH-responsive hydrogels include poly (acrylic acid) and poly(methacrylic acid) hydrogels. Next, temperature-responsive hydrogels, are a type of hydrogel that undergoes reversible swelling or shrinking in response to changes in temperature(Sheng et al., 2015). These hydrogels are made up of polymers that have both hydrophilic and hydrophobic parts, allowing them to respond to changes in temperature. At low temperatures, temperature-responsive hydrogels are typically hydrophilic and swollen, while at higher temperatures, they become more hydrophobic and shrink(Xiao, Isner, Hilt, & Bhattacharyya, 2013). This behavior change can be exploited for various applications, such as drug delivery, tissue engineering, and biosensors. Enzyme-responsive hydrogels can change their physical or chemical properties in response to the presence or activity of specific enzymes(Sobczak, 2022). They can be made from a variety of natural or synthetic materials, including polymers such as PEG, chitosan, and HA. Chemical-responsive hydrogels can undergo reversible changes in their structure, properties, or behavior in response to changes in the chemical environment. The response of these hydrogels can be triggered by various chemical stimuli, such as pH, ionic strength, solvent polarity, or the presence of specific molecules(Bustamante-Torres et al., 2021).

2.3.7. Chain composition.

Another criterion used to classify hydrogels is the composition of the polymer chain. Homopolymeric hydrogels are composed of a single type of polymer chain(Bustamante-Torres et al., 2021). These chains can be either natural or synthetic in origin. These hydrogels can be designed to have a range of properties, such as different degrees of swelling, mechanical strength, and biodegradability, depending on the choice of monomer and the method of synthesis. Homopolymer hydrogels have been used in a variety of applications, such as drug delivery, tissue engineering, and wound healing. Examples of homopolymeric hydrogels include poly(acrylic acid) and PEG hydrogels. Copolymeric hydrogels, e.g. poly (N-isopropylacrylamide-co-acrylic acid) and poly(ethylene glycol)-co-poly(lactic-co-glycolic acid) hydrogels, are composed of two or more different types of polymer chains(Bashir et al., 2020). These chains can be arranged randomly or in a specific pattern. By varying the composition of the polymer chains, copolymer hydrogels can be designed to have unique properties, such as improved mechanical strength, enhanced biocompatibility, and improved drug release kinetics(Z. Li & Guan, 2011). Copolymer hydrogels have been used in applications such as drug delivery, tissue engineering, and biosensors(Wang et al., 2019). . Hybrid hydrogels are composed of two or more different types of polymer chains in combination with other materials, such as inorganic nanoparticles or proteins(Montheil, Echalier, Martinez, Subra, & Mehdi, 2018). They can have improved mechanical strength, enhanced biocompatibility, and improved drug release kinetics compared to single-network hydrogels. Hybrid hydrogels, i.e. PEG hydrogels with incorporated gold nanoparticles and gelatin-methacrylate hydrogels have been used in applications such as drug delivery, tissue engineering, and biosensors(Motealleh & Kehr, 2017). Composite hydrogels are composed of two or more different types of polymer chains, each forming a distinct phase within the hydrogel(Tomczykowa & Plonska-Brzezinska, 2019). These different phases can have diverse mechanical, swelling, and degradation properties. By combining different materials, composite hydrogels can be designed to have unique properties and functionalities, making them a versatile class of biomaterials. Composite hydrogels, e.g. interpenetrating network (IPN) hydrogels and semi-IPN hydrogels. have a wide range of potential applications in fields such as tissue engineering, drug delivery, biosensors, and wound healing(Zoratto & Matricardi, 2018). Crosslinked hydrogels are composed of polymer chains that have been crosslinked to form a 3D network structure. Crosslinking can be achieved through a variety of methods, including chemical,

physical, and enzymatic crosslinking. Examples of crosslinked hydrogels include PA hydrogels and chitosan-alginate hydrogels(Tan et al., 2023).

Applications of hydrogels

Hydrogels are systems with three-dimensional spatial network structures using water as the dispersing medium, which are soft in nature, can maintain a certain shape and have strong water absorption capacity (water content can be as high as 99%)(J. Li et al., 2021). In addition, hydrogels also have good biocompatibility, biodegradability, etc. They are widely used in biomedical fields, such as drug release, medical dressing, gum tissue regeneration, bone repair, etc., which are one of the most promising medical materials in the future.

1. Medical dressing

Trauma and trauma infection are the most frequent cases in hospitals. Medical dressings can act as a protective barrier to cover wounds, absorb wound exudate fluid and help wounds heal(Abdelrahman & Newton, 2011). With good flexibility and biocompatibility, hydrogel dressing absorbs liquid well and can create a moist environment for tissue regeneration, and the slip elastic state of the hydrogel can effectively avoid secondary injury caused by wound adhesion, so it becomes an excellent choice for medical dressing.

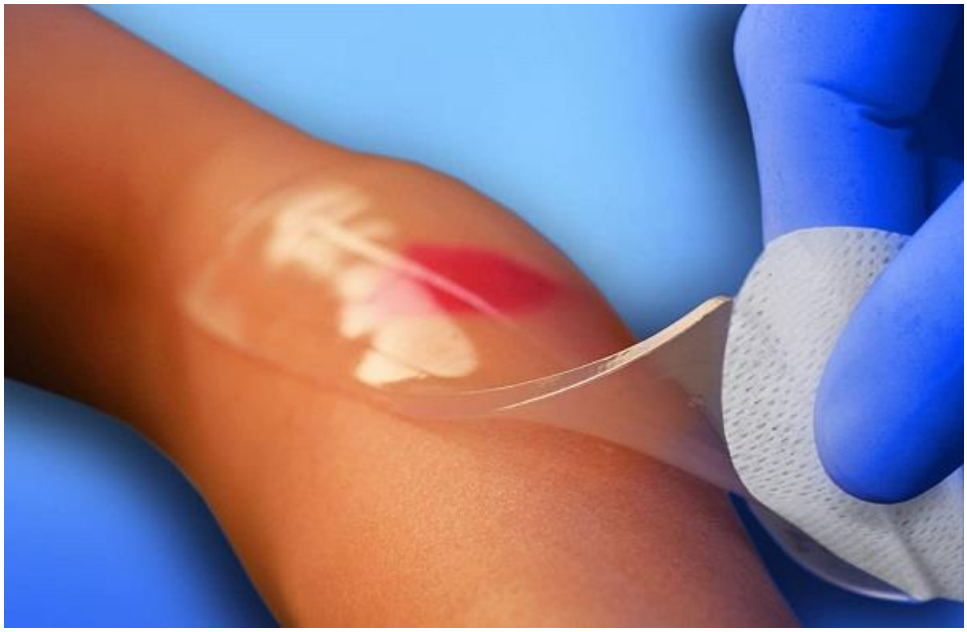

Figure 4 wound dressing

2. Drug delivery

Drug delivery systems are engineered technologies for the targeted delivery and/or controlled release of therapeutic agents(Adepu & Ramakrishna, 2021).

Hydrogels have the functions of storing drugs, controlling the rate of drug release and driving the release, regulating the hardness and strength of formulations as well as promoting decomposition, and masking the odor of pharmaceuticals, which have greater potential for application in the field of drug delivery carriers(Auriemma et al., 2020).

3. **Pulp regeneration**

Pulp regeneration is the application of the principles of tissue engineering, where pulp stem cells are cultured in vitro and implanted on a biocompatible and absorbable degradable biological scaffold to induce pulp stem cells to form pulp-dentin complexes and pulp-like tissues to repair damaged pulp tissue and restore its physiological function(Jung, Kim, Sun, Cho, & Song, 2019).

The hydrogels can be prepared in injectable form and the fluid can be injected to fully fill the pulp chamber and root canal before gelation(Astudillo-Ortiz, Babo, Reis, & Gomes, 2021). After the gelation reaction occurs, the formed hydrogel adheres closely to the surrounding tissue and is well suited for application in pulp regeneration studies. In addition, the hydrogel encapsulates the cells and then undergoes a sol-gel transition, which allows for a more uniform distribution of cells inside the gel and may solve the problem of exogenous cells in pulp regeneration that are difficult to penetrate deep into the scaffold within the root canal(Gong et al., 2009).

4. Cardiac repair

Myocardial infarction is one of the major diseases that threaten human health. After myocardial infarction, a large number of myocardial cells undergo necrosis and form scarring, leading to heart failure(Singh & Jat, 2021). The use of direct heart transplantation for cardiac repair treatment is affected by the number of donors and rejection reactions, with low success rate(Crespo-Leiro et al., 2022). Therefore, cardiac repair using cell transplantation is a more effective method for treating heart failure, but the low retention rate and low survival rate of cells after transplantation greatly limit its widespread clinical application(Zhu & Cheng, 2021).

Hydrogels can be composed of a variety of natural or synthetically derived polymers and assembled into a three-dimensional polymer network with high water content(Varghese,

Rangappa, Siengchin, & Parameswaranpillai, 2020). These characteristics allow hydrogels to mimic the extracellular matrix environment as a vehicle for cell transplantation, promoting cell survival, proliferation, differentiation and migration, and facilitating tissue regeneration. Injectable hydrogels provide a better survival environment for transplanted cells, and in addition to meeting biocompatibility, low immunogenicity, high permeability and tunable mechanical properties, they can be infused into the myocardium via catheter for minimally invasive treatment and are an important component of cardiac tissue engineering.

5. Neural Tissue Repair

Peripheral nerve injury (PNI) causes disruption of axonal continuity, distal nerve fiber degeneration and neuronal death, resulting in loss of motor, sensory and other autonomic functions, which seriously affects patients' normal life(Alvites et al., 2018). The traditional surgical approach to treat PNI is to use autologous nerves and artificial biomaterials for bridging repair, but the limited source of autonomic nerves and mismatch in size limit its application(Vijayavenkataraman, 2020). Tissue scaffolds can be used as a carrier for nerve cell growth and a platform for efficient delivery of neuropharmaceuticals, which can overcome the autologous nerve problem for therapeutic nerve repair(Long, Xie, & Lu, 2022).

Natural polymer hydrogels are a class of highly biocompatible materials that resemble the human extracellular matrix environment and are a hot medical material for neural tissue repair applications in recent years(Jose, Shalumon, & Chen, 2020). Its mechanical properties such as hardness and viscoelasticity are similar to human nerve tissues, and the dense three-dimensional structure constructed after swelling can promote nerve growth, and the hydrogel neural tissue scaffold with drug slow release function can be prepared by carrying bioactive substances. In addition, the natural polymer hydrogel can be used as a cell culture platform to orientate cells, promote nerve axon growth, and compensate for damaged neural gaps, thus repairing peripheral nerve damage(Shen & Xu, 2015).

6. Bone tissue repair

Bone defects caused by aging, disease, trauma and other factors are extremely harmful to the human body, so effective treatment methods are needed to achieve bone tissue repair and regeneration. Bone grafting is an important means of treating bone defects, however, the lack of

bone grafting material sources, unsound donor bone tissue for allogeneic bone grafting, and the risk of infection and complications have limited the application of bone grafting(Cofield, 2007).

Stimulus-responsive hydrogels contain the advantages of ordinary hydrogels and are capable of sensing external physicochemical stimuli such as temperature, light, pH and magnetic field, which in turn trigger transformations in properties such as three-dimensional shape and solid-liquid phase state, resulting in properties such as injectability, self-healing, and shape memory(Roy, Manna, & Pal, 2022). These hydrogels can be used to carry active cells and cell growth factors and implanted into defective tissues for bone tissue damage repair and functional reconstruction.

CHAPTER THREE: MATERIALS AND METHODS

3.1.1 Research design.

The research was carried out in three parts that is extraction of wound healing bioactive components, phytochemical screening of wound healing components and hydrogel formulation and characterization.

3.1.2 Sample collection

The plant materials consisted of the leaves of jatropha multifida. The samples were collected in march in kumi district, kumi sub county in a village called agule to tororo in eastern uganda where the research was conducted. The samples were brought to the laboratory the same day. The experiments were carried out from march to april 2024 in the laboratory of chemistry, faculty of science and education Busitema university.

3.1.3 Materials.

The materials used in this study include, jatropha multifida leaves,mesh sieve, electric grinder, 4 beakers,test tubes, funnel, weighing scale, stirring rod, thermometer, 250ml measuring cylinder, filter papers, glass plates, rotary evaporator, and the main reagents used for laboratory analysis include 99.8% methanol, distilled water, mayer's reagent ferric chloride solution, 1% hydrochloric acid solution.

3.1.4 Treatment of the plant material.

The harvested leaves were washed and then cut into small pieces and dried in a cold drying room (laboratory, 25℃) for about one week, after which time the leaves were brittle and were practically anhydrous. Then the dry leaves were crushed into powder form using electric grinder. The powder thus obtained was sieved with a mesh sieve with diameterof 710µm, thus the fine powder obtained was weighed and the mass recorded in grams. 50g of the fine powder was then placed in a beaker and 300ml of methanol was then added and stired after 30minutes. The mixture was left to stand for 24 hours and then stired and filtered.

The filtrate was kept in different beaker and covered using aluminium foil while 300ml of methanol was added to the residue stired and left for 24 hours and then filtered. This procedure was repeated three times . After the filtrate was kept for further analysis and residue was disposed off.

The filtrate was then concentrated using a rotary evaporator at a pressure of 120rpm and at a temperature of 36℃ for two hours. The viscous concentrate was once again dissolved in 10ml of

methanol, stored in the fume hood for 5 days to allow the solvent to evaporate to obtain the crude extract. The extract was then subjected to phytochemical analysis**.**

3.1.5 phytochemical analysis.

Phytochemical screening was based on the differential reactions (coloration and precipitation) of the main groups of chemical compounds contained in plants. This analysis included:tannins, flavonoids, alkaloids, steroids,terpenoids,saponins, quinones, phenols, mucilage and anthocyanins.

3.1.5.1Test for alkaloids.

1 wagner's test. 1ml of wagner's reagent was addad to 1ml of the acidic crude extract. The formation of a reddish brown precipitate indicated the presence of alkaloids.

3.1.5.2Test for flavonoids.

3.1.5.2.1 Ferric chloride test.

3 drops of neutral ferric chloride solution was added to 1ml of an alcoholic solution of crude extract in a test tube. The formation of blackish red colour indicated the presence of flavonoids.

3.1.5.2.2 lead-acetate test.

3 drops of aqueous lead acetate solution was added to 2ml of the alcoholic crude extract in a test tube. The appearance of reddish-brown precipitate indicated the presence of flavonoids.

3.1.5.3 Test for tannins.

Ferric chloride test. 3 drops of ferric chloride solution was added to 1ml of the alcoholic solution of the crude extract in a test tube. The formation of blackish precipitate indicated the presence of tannins.

3.1.5.4 Terpenoids test.

To 2ml of an alcoholic solution of the crude extract in atest tube was added 1ml of acetic acid followed by 1ml of chloroform,then concentrated sulphuric acid was added to the mixture. The absence of expected blue-green colouration indicated the absence of terpenoids.

3.1.5.5 Test for saponins. 5ml of the pant extract was put in a boiling tube and boiled with distilled water, formation of stable form indicated the presence of saponins.

3.1.5.6 Test for quinones.

3ml of an alcoholic solution of crude extract in a test tube was added 3ml of alcoholic potassium hydroxide solution. The absence of expected red to blue coloration indicated quinones were not present.

3.1.5.7 Test for steroids.

Lieberman's test. 1ml of the crude extract in a test tube was added 1ml of acetic anhydride and then cooled in ice cold water. While still in ice cold water the mixture was treated separately with, 20% ice cold sulphuric acid, ice cold 50% sulphuric acid and finally wit ice cold 98.8% sulphuric acid. The expected colour change from violet to blue was not observed.

3.1.5.8 Test for phenols.

Ferric chloride test. 1ml of ferric chloride solution was added to 3ml of an alcoholic crude extract in a test tube followed by distilled water. The formation of green-blue coloration indicated the presence of phenolic hydroxyl group.

3.2 preparation and development of the hydrogel.

3.2.1 Materials.

Chitosan pharmaceutical grade with 85-93% degree of acetylation was purchased from the market. Acetic acid glacial and gelatin were purchased from batala dealers in reagents and chemicals mbale city. Distilled water was obtained from the chemistry Laboratory: busitema university, Nagongera campus

3.2.2 Preparation of hydrogel and extract solution

Beforehand, chitosan, gelatin, and jatropha multifida leaf extract were prepared as the solutions. Chitosan solution was prepared by dissolving 0.25g of chitosan in 25ml of 2.5 % v/v acetic acid solution by stirring using magnetic stirrer. Gelatin solution was prepared by dissolving 5g of gelatin in warm water (60℃) and mixed by keeping the solution temperature minimum at 40℃ to avoid gelation. And the solution of extract was prepared by dissolving 5g in 5ml of dimethylsulphuroxide (DMSO). The chitosan solution, the gelatin solution, and the extract solution were mixed. Water was added to the mixture to give the final volume of 100 mL. Then the mixture was stirred thoroughly at 40℃. The resulting solution was cast on a glass plate by 2 – 4 mm in thickness.

Finally, the hydrogel was dried in the oven at 38℃ overnight. Some hydrogels have been prepared by various proportions of chitosan, extract, and gelatin, as shown in Table 2.

Entry	1	2	3	4	5	6	7	8	9
Chiosan (g)	0.5	0.35	0.5	0.5	0.5	0.75	0	0.05	1
Gelatin (g)	10	5	15	15	10	20	5	5	20
Extract (ml)	5	5	10	5	10	0	5	5	15

Table 2 chitosan, gelatin and extract composition for hydrogel preparation

3.2.3 Gel fraction determination. The determination of the gel fraction was conducted by a gravimetric method. The hydrogel was cut into small pieces and dried in the oven at 60℃ until it reached a constant weight (W0). Then, the dried hydrogel was immersed in water and shooked for 24 hours at room temperature. Finally, the hydrogel was re-dried in the oven at 60℃to constant weight (W1).

The gel fraction was calculated using equation 1.

$$\text{Gel fraction } = \frac{w_1 - w_0}{w_0} x100\%$$

3.2.4 Swelling index determination. The hydrogel was dried in the oven at 37℃ overnight. The dried hydrogel weight was recorded as W0. Then the hydrogel was immersed in distilled water at room temperature. The hydrogel was weighed (Wt) every 30 minutes during 120 minutes. Then, the swelling index was calculated using Equation. 2

$$\text{Swelling index } = \frac{w_t - w_0}{w_0} x100\%$$

3.3 microbial activity.

3.3.1 Microbial strains.

Strains of Gram-positive cocci such as staphylococcus aureus and Gram-negative baccili such as Escherichia coli, isolated from skin infections in the microbiology laboratory of Busitema

university, nagongera campus were used for anticrobial testing. They were stored in agar-based nutrient media at 4℃ before use.

3.3.2 Preparation of the medium for bacteria growth

Step 1: 52g of the medium (MacCONKEY) was suspended in one litre of distilled water.

Step 2: The solution was then boiled to dissolve it completely and then it was allowed to cool for 30 minutes.

Step 3. The solution was then transferred into durnren bottle and sterilized by auto-cleaving at 120℃ for 15 minutes. And this helped to kill any microbes that were present in the medium

Step 4: the medium was then placed into a sterilized petri dish

The solution was allowed to settle and then inoculations was carried out (microbes were introduced into medium), The suitable environment was provided for the inoculated microbes.

Bacterial assay was carried out using disc diffusion method to determine the capacity of the extract to inhibit bacteria growth at a dose of 3mg/ml. The mixture was then incubated for 48 hours, after incubation period (37℃ for 48h), the dishes were examined for inhibitory zones.

CHAPTER FOUR. RESULTS AND DISCUSSION.

4.1 Phytochemical screening .

The results of the phytochemical screening carried out on the leaves of jatropha multifida plant are recorded in the table 2 below.

Jatropha multifida leaves were rich in secondary metabolites. The tests indicated the presence of alkaloids that can be toxic at high doses. In addition it contained saponins, phenols, flavonoids, saponins and tannins. However, steroids, terpenoids and quinones were absent.

Chemical group	Staining
Tannins	+
Flavonoids	+
Alkaloids	+
Steroids	-
Terpenoids	-
Phenols	+
Quinones	-
Saponins	+

Table 3 Results of phytochemical tests: +presence, - absence

4.1. 2 Efficacy of jatropha multifida leaf extract.

The extract was was ineffective on the strains of staphylococcus aureus and Escherichia Coli.

The failure of j. multifida leaf extract to reach the minimum inhibitory concentration (MIC) required to effectively inhibit the bacterial growth was as a result of the following factors.

Insufficient concentration of the active compounds. J. multifida leaf extract contained various bioactive compounds such as, alkaloids, phenols, flavonoids and others that may have antibacterial properties. The concentration of these compounds was too low that did not reach the MIC required to kill the bacteria or effectively inhibit the bacterial growth. Another factor could be variability in extract composition. The composition of the plant extract can vary significantly based on factors suc as plant species, growth conditions, extraction methods and seasonal variations. Variation in composition can affect the extract's efficacy against the bacteria. It could also be as a result of resistance mechanism of bacteria. Bacteria like staphylococcus aurues and E.Coli can develop resistanceto antimicrobialagents over time. The bacetia developed resistant mechanisms against

the specific compounds present in jatropha multifida leaf extract, this could also be a possible reason why the extract did not inhibit the bacterial growth.

4.1.3 Hydrogel formulation.

Hydrogels have gained a great interest in biomedical research and applications. They can retain water in its three-dimensional network which capable of deforming as soft wet material with the structure that resembles extracellular matrix. Hydrogels can respond to various external stimuli which comprises of pH, chemical composition, temperature, pressure, and electrical field. These properties route the hydrogel application, especially for delivery drug system, wound dressing, and tissue engineering. Herein, I prepared gelatin/chitosan/ jatropha multifida leaf extract hydrogel through a simple physical mixing process at low temperature. Chitosan was previously dissolved in 2.5 % of acetic acid to turn into chitosan polycation. Chitosan polycation and gelatin was dissolved in warm water (which tend to form polyanion), were cross-linked by polyelectrolyte complexation (PEC) process to form 3D networking hydrogel. The proposed chitosan-gelatin interaction to form hydrogel is illustrated in Fig. 5. The presence of ammonium ($NH3^+$) and hydroxyl (-OH) groups of chitosan results in hydrogen bonding with the amine (-NH-) and/ carboxyl (-COOH) groups of gelatin. The ionic bond between the ammonium groups ($NH3^+$) groups of chitosan and ionic carboxyl (-COO-) groups of gelatin. All those interactions result in a high order 3D structural bi-polymer system.

The resulting gelatin/chitosan/ jatropha multifida leaf extract hydrogels have transparence soft film sheet with 2 - 4 mm thick.

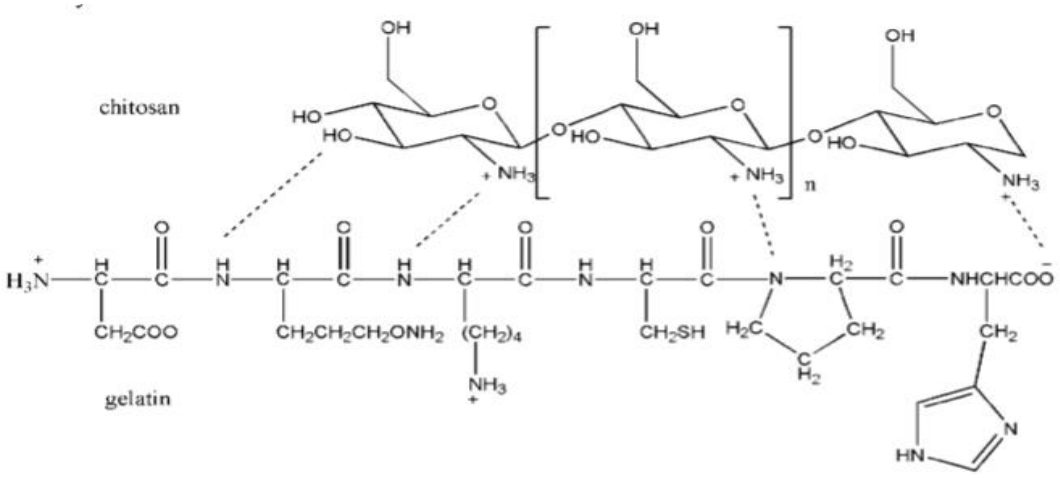

Figure 5 The possible interaction between chitosan polycation and gelatin polyanion in the formation of three-dimensional network of the hydrogel

Figure 6 The resulting gelatin/chitosan/jatropha multifida leaf extract hydrogel film sheet

4.1.4 Effect of gelatin mass.

Effect of gelatin mass. Gelatin is commonly composited with chitosan to prepare a hydrogel through a polyelectrolyte complexation (PEC) mechanism. Herein, the effect of various gelatin quantities to the gel fraction of the as-resulted hydrogels was firstly evaluated. The gel fraction implies the cross-linked degree of the polymeric chain of bi-polymer gelatin and chitosan. shows that the hydrogels with a better gel fraction have been resulted in by the gelatin/chitosan ratio of 20:1 and 40:1. However, the hydrogel with higher gelatin/chitosan ratio shows a lower gel fraction. According to the illustration in Fig 5, the possible most potent interaction between chitosan and gelatin could be the ionic bond between the $NH3^{+}$ of chitosan and $–COO^{-}$ of gelatin. A higher gelatin/chitosan ratio may hide the chitosan as the polycation source, hence lowers ionic bond and reduces crosslink order. The effect of gelatin composition toward the swelling index of the gelatin-chitosan-based hydrogels was also investigated. Swelling index implies the capability of the hydrogel to absorb and retain water in its 3D structure. Water is bound inside by hydrogen bonding. Therefore, swelling index correlates with the presence and the orientation of many functional groups that responsible for hydrogen bonding such as carboxyl (-COOH), amine (-NH-), and hydroxyl (-OH), which present both in chitosan and gelatin. High swelling index may represent that many hydrogen bonds occurred. Herein, the best swelling index behavior was resulted in by

20 g of gelatin in composition. This result also comparable with the other work. The swelling index at higher gelatin content was getting lower, which probably correlates with the decrease of gel fraction, as explained above.

4.1.5 Effect of chitosan mass.

It is well known that gelatin has an excellent filmogenic as well as gelation properties.

Therefore, the hydrogel, composed of gelatin and extract without chitosan, shows a gel fraction of almost 60 % and a swelling index of 300 %. Gelatin has a molecular structure which enables to form a zwitterionic system that stimulates cross-link. The high swelling index of gelatin hydrogel corresponds to the abundance of functional groups for hydrogen bond interaction. The addition of chitosan has improved the hydrogel quality by increasing the gel fraction up to almost 70 %. The interaction polycation chitosan and polyanion gelatin can lead to the development of high order crosslink structure as previously explained. The swelling index also increases from 300 % to 400 % progressively with the mass of chitosan added. This improvement can be generated due to the presence of chitosan automatically increases the hydroxyl groups (-OH) that retain more water by hydrogen bonding in the hydrogel structure.

CHAPTER FIVE : CONCLUSION AND RECOMMENDATION.

5.1 CONCLUSION.

The results from this study showed that the leaf extract of jatropha multifida contained alkaloids, flavonoids, phenols, tannins,and saponins. Therefore the presence of these bioaactive phytochemicals shows that the plant can be used traditionally for treatment of wounds, anti-inflammatory, anti-influenza and others. Though the result of the antibacterial of the extract was ineffective, this was as a result of the reasons discuused before.

In conclusion, phytochemical screening of the leaf extrac of j. multifida and the formulation of gelatin-chitosan hydrogel loaded with the extract could offer a promisisng mechanism for the management of wounds.

5.2 RECOMMENDATIONS.

I recommend that quantification analysis should be done in order to investigate the total content of secondary metabolites contained in the powdered leaves of j. multifida also I recommend further bacterial assays should be done,testing the plant extract with other bacteria using different methods to investigate the antimicrobial activity of the extract. More work should also be done on testing the antimicrobial activity and the wound healing properties of the hydrogel and furter studies should be done on extraction methods,doing serial extraction with different extaction sp

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