International Journal of Environment, Agriculture and Biotechnology
Vol-8, Issue-6; Nov-Dec, 2023
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

Temperature and pH-sensitive chitosan-collagen hydrogels for antimicrobial and wound healing applications

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Received: 10 Nov 2023; Received in revised form: 11 Dec 2023; Accepted: 18 Dec 2023; Available online: 26 Dec 2023

Abstract— *Hydrogels have proven to be of great value because of their useful properties and functions. Temperature and pH-sensitive chitosan-collagen-based hydrogels have drawn research interest in the area of wound care. Their unique mechanism allows them to release antimicrobial agents in response to changes in the environment and aids in efficient healing of wounds. The current review aims to describe various composite hydrogels of chitosan and collagen, their temperature and pH sensitivity, the antimicrobial effect of chitosan-collagen hydrogels and their applications in wound healing. Sources and properties of chitosan and collagen have also been stated in order to highlight their importance in these hydrogels. The main focus revolves around the potential of chitosan-collagen hydrogels as antimicrobial biomaterials for healing wounds in an efficient manner.*

Keywords— ***Antibacterial, hydrogels, pH, temperature, wound healing***

I. INTRODUCTION

Hydrogels' unique properties make them essential vehicles for specific medications. This is because of their well-known capacities to keep the wound interface moist, suppress infectious activity, eradicate surplus exudates from the wound, encourage wound healing, and have a reasonable level of biocompatibility with cell tissues (Vaneau et al., 2007). Hydrogel dressings are therefore recommended for wound healing (Li et al., 2016). Moreover, it is crucial to incorporate biocompatible macromolecular components into degradable hydrogel wound dressings (Jie Zhu et al., 2019).

Hydrogel dressings can be used to keep a wound wet and absorb tissue exudates. Next, as the wound area is cooled, the dressing allows oxygen to permeate and provides pain relief (Dong et al., 2014). Finally, the usage of hydrogel dressing was more strongly linked with the characteristics of in-situ encapsulating medicines, wound sites filling, and adhesion to wounds (Tran et al., 2011). Similar to how hydrogels work as barriers against microbes, using hydrogel as a wound dressing has been a popular choice in studies.

They also provide 3-D structures for cell adhesion and development and preserve wet surroundings at the wound interface (Kokabi et al., 2007). The polymer chitosan is derived from chitin, which is the second most abundant polymer in nature. It exhibits remarkable qualities such as making it an indispensable biomaterial that is attracting significant industrial interest as a potential replacement for synthetic polymers in the future (Kurakula, 2020).

All of the body's numerous connective tissues, such as the skin, bones, ligaments, tendons, and cartilage, include

collagen, the most prevalent structural protein. Collagen is another essential element in the wound-healing process. Collagen is essential for every stage of wound healing, including hemostasis, inflammation, proliferation, and (Liu et al., 2019). Collagen serves as the natural structural foundation for the synthesis and growth of new tissue. Collagen, the most structural protein found in the extracellular matrix of the body's connective tissues, including skin, bones, ligaments, tendons, and cartilage, is crucial for wound healing. Collagen is essential for all phases of wound healing, including hemostasis, inflammation, proliferation, and remodeling. It acts as a natural structural basis for the development of new tissue (Gu et al., 2019).

In hydrogels, there exist both synthetic and natural polymer chains (Ahmad et al., 2022). Agarose, alginate, chitosan, collagen, gelatin, hyaluronic acid, and cellulose derivatives are examples of natural polymers frequently employed in hydrogel formulations (Gasperini et al., 2014)). These polymers are useful for biological applications because of their inherent biocompatibility and biodegradability. It is possible to create hydrogels with certain properties by adjusting the chemical and physical properties of synthetic polymers (Almajed et al., 2022). To join the polymer chains and create the hydrogel's network structure, crosslinking agents are used. These substances might be chemical or physical. Physical crosslinking, including reversible connections like hydrogen bonds or physical entanglement, can be brought on by changes in pH, temperature, or other environmental factors. Covalent bonds between polymer chains are formed via chemical crosslinking, which is commonly accomplished through chemical processes like condensation or free radical polymerization (Ertl et al., 2020).

Here, we will review about pH and temperature sensitive chitosan and collagen based hydrogel systems and explore their properties with different natural and synthetic polymers by using different crosslinking methods while simultaneously discussing the application of antibacterial and wound healing along with other biomedical materials that the hydrogels can be used with some recent advancements.

II. PROPERTIES OF CHITOSAN AND COLLAGEN AS BIOMATERIALS

2.1 Chitosan

Chitosan is produced by partially deacetylating naturally occurring insoluble chitin (Martínez-Ruvalcaba et al., 2007) found in the exoskeletons of insects, fungi (Merzendorfer, 2011), and crustaceans (Khor & Lim, 2003). Because of the hydrogen interactions between hydroxyl and acetamide groups, chitin has a hard crystalline structure. A higher concentration of amino groups and improved water solubility are obtained when chitin undergoes partial deacetylation and transforms into chitosan. Chitosan deacetylation is increased proportionately, and biocompatibility and biodegradability are improved (Murakami et al., 2010).

2.1.1. Sources of chitosan

Although other species including lobster, crayfish, and oysters have also been used, prawns and crabs are the most frequently mentioned sources (**Fig. 1**) in the literature when discussing the raw materials used to prepare chitosan (Rizeq et al., 2019). The weight percentage (wt%) of chitin varies depending on the organism. For example, the waste from crustacean shells typically contains 30% to 50% calcium carbonate and 20% to 30% chitin. However, in certain lobster genera, like Nephrops sp. and Homarus sp., the chitin content of the shells ranges from 60% to 75%, which is the highest of all chitin-containing species (Arbia et al., 2013).

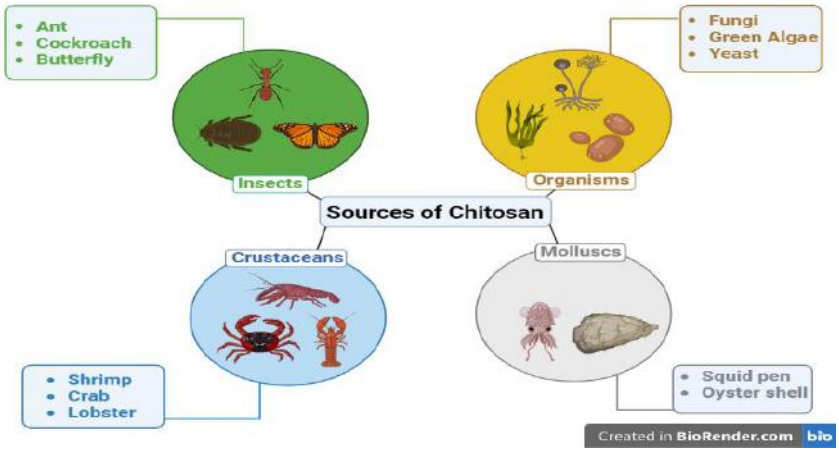

Fig. 1: Sources of Chitosan

Studies that have already been conducted on the extraction of chitin or chitosan from crustacean byproducts that contain 20% (wt%) or more of chitin have produced encouraging results when used as industrial feedstocks for the synthesis of chitosan. For example, Procambarus clarkii (crayfish) by-products, which included the entire animal body, thorax, and claws, have been found to contain roughly 20% to 23% (by weight) of chitin. Because this source is readily available and inexpensive, it already justifies its use as an economically viable source for chitin production on an industrial scale (Bautista et al., 2001).

The economic and environmental benefits of using these crustacean sources for chitosan preparation have also been suggested by previous research. This is because 40–50% of the mass of crustaceans that are harvested for human consumption is wasted, and the majority of this waste is disposed of in the sea, where it causes serious pollution (Vázquez et al., 2013). Consequently, it is possible to identify byproducts of crustacea, such as lobster cephalothorax, as a good source for the industrial synthesis of chitosan.

2.1.2. Chemical structure of chitosan

Chitosan (CS) is a naturally occurring cationic polymer that is derived from chitin through a process of alkaline deacetylation **(Fig. 2)**. It shares structural similarities with glycosaminoglycan, a component of the extracellular matrix (Raj et al., 2018). Covalent bonding between the chitosan macromers results in chemically cross-linked hydrogels; this bond formation is irreversible. Chitosan cross-linked system, hybrid polymer networks (HPN), interpenetrating polymer networks (IPN), and semi-interpenetrating polymer networks (SIPN) are the four states of creation of chemical cross-linked hydrogels. The second chain in the derivation may resemble or differ from the first structural unit (Shi et al., 2018). Chemical cross-linking is caused by hydroxyl groups and amines on chitosan chains. Chemical cross-linking can happen through photopolymerization reactions or cross-linkers (Fidalgo et al., 2018).

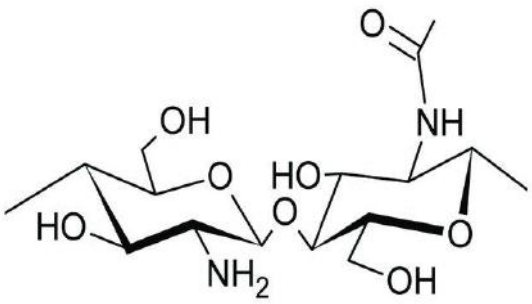

Fig.2: Chemical Structure of Chitosan

It is common to see hydrogen bonds form between the functional groups of chitosan and polyurethane chains. In order to improve the interaction between the two components, this is significant (Mohraz et al., 2019). The FTIR spectra of the composites studied confirmed the presence of distinct absorption bands for PUR and Chit (Qin & Wang, 2019). The addition of Chit to the PUR matrix resulted in a shift in the C=O wave number from about 1700 cm-1 to 1640 cm-1, as well as an increase in the band of –O–H and –N–H stretching vibration at around 3500 cm-1 to 3400 cm-1. These unambiguously showed that PUR and Chit in the third composite formed hydrogen bonds (Hernández-Martínez et al., 2017).

FTIR scans showed a comparable shift in the carbonyl band to a lower wavenumber as Chit in composites increased (Gupta & Kim, 2019) **(Fig 3)**. The study analyzed six composites with varying chitosan flakes percentages, revealing a uniform nanoflake structure. Monitoring changes in polyurethane structure was challenging due to similar functional groups. The absence of a urea peak in FTIR spectra suggests no secondary reaction or covalent bond formation (Garnica-Palafox & Sánchez-Arévalo, 2016).

It is possible to insert chitosan into the polyurethane framework after lowering its size to the nanoscale, as verified by FTIR (Gupta & Kim, 2019). Even if there isn't a distinct continuous phase (matrix) and filler, the materials that were created were nevertheless referred to as biocomposites. Nevertheless, compared to traditional composites, the material's trend of changing properties is distinct. Adding chitosan to the polyurethane framework increased the material's mechanical strength by increasing cross-linking. ATR-FTIR analysis of eight composites revealed that PUR chains were present on the surface of the samples, while Chit particles were submerged in the bulk of the matrix (Brzeska et al., 2019).

2.1.3. Properties of chitosan

Chitosan is a naturally occurring polysaccharide that is renewable, non-toxic, biodegradable, and biocompatible (Croisier & Jérôme, 2013). The solubility of chitosan is attributed to the protonation of the amino group (NH2) on its chains into a positively charged group (NH3 +) at pH values lower than its pKa (pH < 6.2). Numerous applications, including tissue engineering, drug delivery, water treatment, biosensors, and water treatment (Teotia et al., 2015), are made possible by soluble chitosan (Kim & Kim, 2017). Chitosan amino groups can be neutralized to produce physical chitosan hydrogels (Ladet et al., 2008). Nevertheless, chitosan has a low mechanical strength, particularly when it is wet (Latza et al., 2015).

3D printing of a chitosan physical hydrogel was described to construct the structure on a three-axis positioning platform. This involved directly depositing the ink in air **(Fig. 4)**. By dissolving chitosan in an acidic combination, a low concentration (8 wt%) 3D-printable chitosan ink was created. This ink was directly converted into three-dimensional scaffolds layer by layer by solvent evaporation after being extruded through micronozzles (Wu et al., 2017). The inks' rheological characteristics are crucial to the extrusion-based 3D printing techniques. Researchers looked on apparent viscosity related to the process in order to find the right concentration of polymer for solvent-assisted 3D printing (Guo et al., 2013).

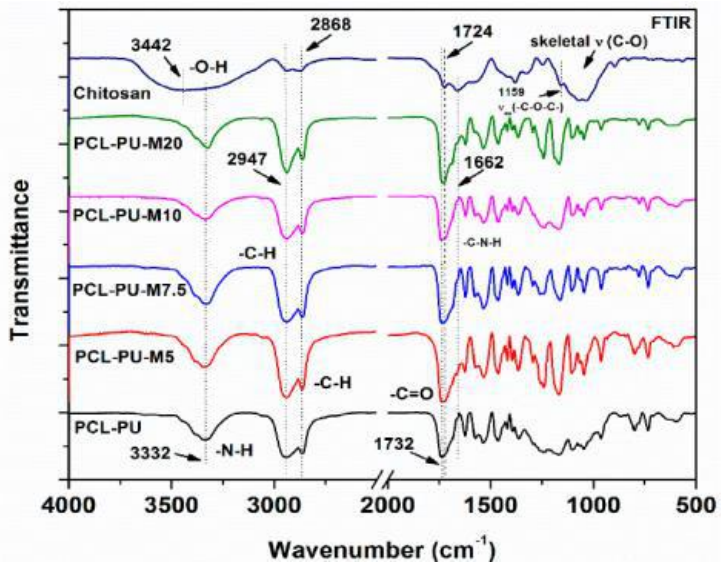

Fig.3: Chitosan FTIR Spectra with 6 composites

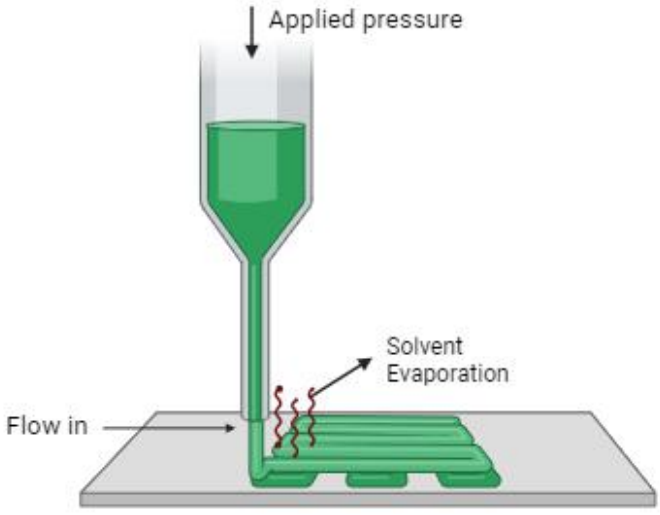

Fig.4:3D printing process diagram and key process parameters

A wide range of investigations have been undertaken to examine the rheological properties of solutions derived from chitosan, encompassing physical chitosan hydrogel, thermosensitive chitosan-glycerol-phosphate solutions, and chitosan blends (Cho et al., 2005). Scientists have been examining chitosan closely to solve a variety of problems. Chitosan is a polysaccharide derived from chitin by the process of deacetylation (Ali & Ahmed, 2018). It is characterized by its relatively low cost and high yield. It is the second most abundant natural biopolymer (Tripathy et al., 2018). However, because of their excellent biodegradability, biocompatibility, immunogenicity, and low toxicity, chitosan hydrogels have previously been used in a wide range of disciplines, including material science (Fu et al., 2016) and biomedicine (G. Chen et al., 2017). Thus, chitosan has been the subject of numerous prior research and among the several techniques that have been suggested, solubilizing chitosan in an acidic aqueous media has historically been the most widely used technique for creating chitosan hydrogels (Bhattarai et al., 2010).

Nevertheless, the limited mechanical properties of chitosan hydrogels impose restrictions on their potential uses (Wang et al., 2016). Several approaches have been employed thus far to enhance the of chitosan hydrogel's mechanical properties (Cao et al., 2018). These methods encompass chemical crosslinking, incorporation of nanofillers possessing superior mechanical properties, and blending with other polymers (Li et al., 2018). While there are moderate enhancements in the mechanical qualities, the inherent characteristics of chitosan are compromised. Moreover, it was discovered that several of the crosslinking agents exhibited toxicity or resulted in undesirable interactions with the bioactive components (Oryan et al., 2018). Consequently, improving mechanical properties of chitosan hydrogel has emerged as a prominent area of research. A unique solvent system was proposed by utilizing alkali-urea aqueous solutions for the synthesis of chitosan hydrogel. This approach resulted in notable improvements in both hardness and toughness, all achieved without the need of any cross-linking agents (Cao et al., 2018).

Alkali-urea solution system was employed for the synthesis of chitosan hydrogel. The research incorporated Ag nanoparticles as both a filling and secondary reinforcing material in order to enhance the mechanical properties of chitosan hydrogel. The objective was to develop a chitosan hydrogel with exceptionally high mechanical properties (Zhao et al., 2018). The primary design principle is utilizing the amino groups present in chitosan as a chelating agent for Ag ions through coordination interactions, hence enhancing the mechanical strength. The secondary objective of incorporating Ag nanoparticles into the chitosan hydrogel is to enhance its antibacterial properties, hence mitigating the risk of wound infection (Fan et al., 2014). Silver is a frequently utilized antimicrobial agent owing to its broad-spectrum antibacterial activity against aerobic and anaerobic microorganisms (Wang et al., 2018).

Furthermore, chitosan possesses the advantageous characteristic of being a biocompatible polyelectrolyte. This property enables it to function as a stabilizing ligand, so restricting the toxicity of silver metal while yet permitting its antimicrobial activity for infection control purposes (Fan et al., 2014). Subsequently, varying proportions of Ag ions are introduced into the structured hydrogel to form chelation complexes with chitosan (Yi et al., 2003). The reductive silver nanoparticles were incorporated into the system using trisodium citrate as a green reducing agent. The objective was to create a chitosan-Ag nanoparticles hydrogel that would exhibit enhanced mechanical properties, increased antibacterial activity, and accelerated wound healing abilities. The hydrogels were subjected to a comprehensive evaluation of their mechanical properties, swelling characteristics, antibacterial activity, and wound healing impact which were found to be satisfactory (Thomas et al., 2007).

2.1.4. Role of chitosan in wound healing

Extensive research has been conducted on chitosan-based materials, with particular focus on wound dressings, due to their diverse range of properties such as antibacterial, anti-inflammatory, and biomedical applications. The antibacterial and anti-inflammatory properties of chitosan have shown promise in the field of wound healing, positioning it as a potential biomaterial (Ahmed & Ikram, 2016). Optimal qualities tailored to specific wound types, cost-effectiveness, and minimal patient inconvenience are key considerations for the development of effective dressings. Chitosan has various applications in multiple domains along with concurrent researches in the field of wound healing. Chitosan has antimicrobial properties which makes it a suitable material for clinical utilization and biomedical applications. It has an important role in wound healing procedures. Wound healing is enhanced by chitosan and synthetic/natural polymer scaffolds. The utilization of chitosan-based scaffolds immobilized with oil for wound healing purposes is now under investigation. Drug loaded scaffolds with chitosan as a base material for wound healing is an area of interest around the world. Chitin and chitosan are known to stimulate wound healing, with research showing their potential to expedite the process. These materials are used in various forms, including nano fibers, gels, scaffolds, membranes, filaments, powders, granules, sponges, and composites. Their primary biochemical functions include

activating polymorphonuclear cells, stimulating fibroblast activity, creating cytokines, migrating giant cells, and simulating the synthesis of type IV collagen (Mezzana, 2008).

Nano-fiber matrices are promising for tissue engineering in skin substitutes due to their ability to facilitate oxygen diffusion, high porosity, diverse pore sizes, and morphological resemblance to the skin's extracellular matrix. These properties enhance cell adhesion, migration, and proliferation **(Fig. 5)**. Progress in chitin and chitosan nanofibril materials has also improved flexibility and utility for creating novel bio-related products. The field of process chemistry has made significant progress in producing these materials (Mattioli-Belmonte et al., 2007).

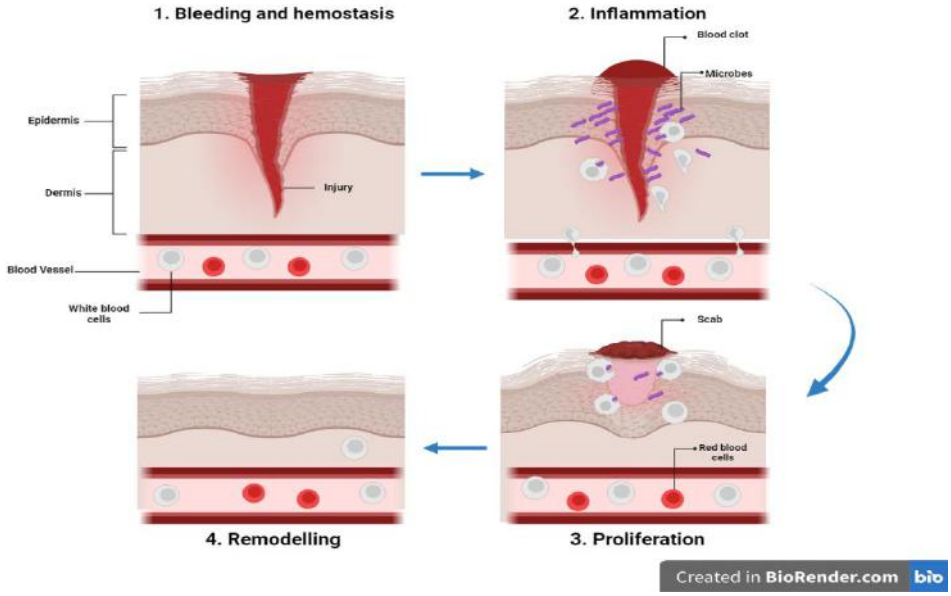

Fig.5: Mechanism of wound healing with diagram

2.2. Collagen

Collagen is a highly prevalent protein synthesized within the human body. The process under consideration is responsible for maintaining the integrity and strength of bodily tissues through the formation of supportive networks that span across cellular structures. Over time, the structural integrity of the fiber deteriorates, resulting in several consequences, one of which is the development of wrinkles on the skin (Chang et al., 2012). It has been empirically demonstrated that the consumption of hydrolyzed protein can facilitate the replacement of damaged fibers with new ones. Consequently, the promotion of collagen creation ensued, so facilitating the process of healing and enhancing the overall appearance of the tissue (Schagen, 2017).

Collagen, as reported by the Protein Data Bank, is the predominant structural protein found in the human body. It provides essential support to a variety of tissues, including tendons, skin, and teeth (with collagen being connected to mineral crystals). Collagen fibers are frequently observed to possess a white coloration, exhibiting an opaque appearance, and are easily identifiable within various tissue samples. Collagen is classified as a viscoelastic substance, characterized by its notable tensile strength and limited extensibility. It is widely recognized for its low immunogenicity, hence reducing the likelihood of rejection whether administered orally or by injection into a foreign entity. The fractions that are capable of inducing an immunological response are specifically situated inside the helical area of the chains and the telopeptide region (Kumar et al., 2014).

2.2.1. Sources of Collagen

Collagen, a naturally occurring polymer present mostly in fibril forming proteins within the cartilage, bone, tendon and skin, exhibits versatile applications in several industries such as food, cosmetics, and medicines (Wang et al., 2013). The extraction of commercial collagen from several wild species has been extensively investigated because to its significant economic value. While it is true that mammals possess collagen protein, non-mammals are also of significant interest due to their likeness to the human body and their potential as abundant economic resources. Collagen is sourced from several origins, encompassing fish, avian species, bovines, marine organisms, kangaroo tails, chicken feet, horse tendons, frog bones and skin, rat-tail tendons, sheepskin, and sporadically even human sources (Silvipriya et al., 2015). However, the use of pig skin for collagen extraction is prohibited in certain countries with a mostly Muslim population (Wang et al., 2014). Since the 1930s, pig skins and bovine have been the primary sources of collagen in commercial applications, while fish

scales are used in religious practices to reduce exposure to infections. Research has been conducted to find alternative collagen sources, with the industry focusing on mammalian collagen. Type I collagen from fish scales has similar properties to mammalian collagen, and marinated or salted skins have lower protein content compared to cold-water fishes (Rawat et al., 2021).

Collagen utilized in industrial settings primarily comes from mammalian sources, like pig and cow collagen. Since these collagens are associated with several problems, such as an outbreak of foot-and-mouth disease (FMD), an outbreak of bovine spongiform encephalopathy (BSE), and religious prohibitions, it is imperative to find a new source (Duan et al., 2009). Numerous marine species have been identified as potential safe and alternative sources for collagen extraction. These species include eels, cuttlefish, seaweed pipe fish, squid, catfish, and ocellate puffer fish (Veeruraj et al., 2013).

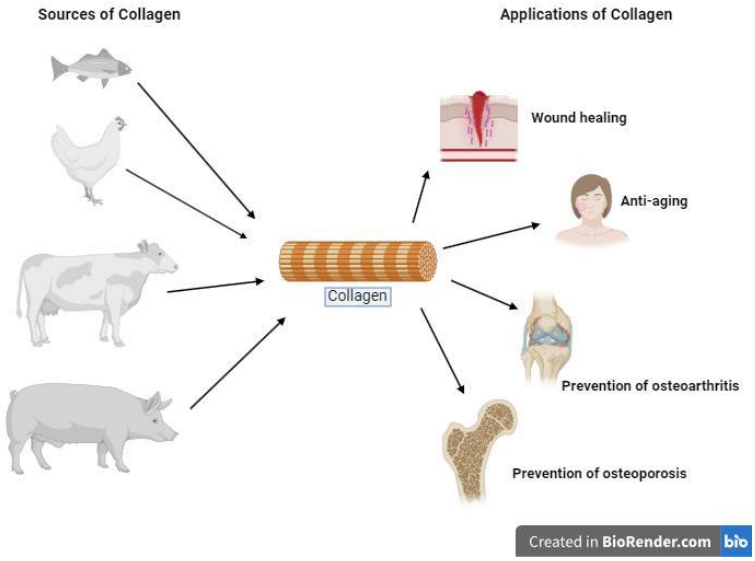

Fig. 6: Sources and Applications of Collagen

In addition, compared to collagen from mammals, that from marine creatures is less immunogenic, less poisonous, and has a higher collagen concentration. The skin, scales, bones, and fins of freshwater and marine fish species are the primary suppliers of collagen. Furthermore, skin, scale, and bone—all of which have a high collagen content—make up almost 30% of the wastes produced by the fishing industry during the processing of fish (Wang et al., 2008). Fish skin typically has a lot of collagen that can be harvested and utilized (Liu et al., 2008). The finest solutions for effective waste management and the creation of value-added goods that boost the fishing industries' profits may be found in the environmentally friendly extraction of collagen from these wastes (Kittiphattanabawon et al., 2005). Fish collagen has a number of drawbacks, including low mechanical strength, low amino acid content, quick biodegradation, and melting point. Fish collagen can be functionally modified and combined with other synthetic or natural polymers to solve these issues (Subhan et al., 2015).

There are many other sources of collagen, but at the moment, animal collagen is the most common type used in collagen products, the majority of which are made from raw materials like cow's milk (Silvipriya et al., 2015). Numerous skin fragments from pigs and cows are used to make the majority of collagen products. Collagen makes up up to 85% of the protein in tendons and makes up one-third of the protein mass in cows. Immunological, zoonotic, and religious sensitivities restrict the use of collagen obtained from animals (Kisling et al., 2019). To prevent FMD (foot and mouth disease) and mad cow disease, researchers are working to find a safer source of collagen. In contrast to the adverse inflammatory and immunological reactions in terrestrial animals as well as pandemic health problems, research is being done on marine sources (Maschmeyer et al., 2020). There are many other sources of marine collagen, such as various marine sources, particularly microalgae, and marine vertebrates. At the moment, marine fish, jellyfish, sponges, and other creatures are the primary subjects of collagen extraction research from marine organisms for tissue engineering biomaterials. Collagen is abundant in the epidermis, muscles, and cartilage tissue of these aquatic creatures (Smith et al., 2023).

The amino acid composition and biocompatibility of collagen derived from marine sources are similar to

those of traditional terrestrial mammalian collagen. However, marine collagen offers additional benefits over mammalian collagen, such as easy extraction, abundant sources, which are particularly useful in the fishing and fish processing industries (Ge et al., 2020). Fish are actually widely available, have no religious limitations, and pose little risk of spreading disease, which makes their skin an excellent choice for type I collagen extraction (Lim et al., 2019). Interestingly, it has been observed that certain collagens derived from marine sources can denature at temperatures below the typical physiological temperature of humans. Some collagen-derived biomaterials are practically challenging because of this thermal instability, primarily when employed for human medical purposes (Felician et al., 2018).

2.2.2. Chemical structure of collagen

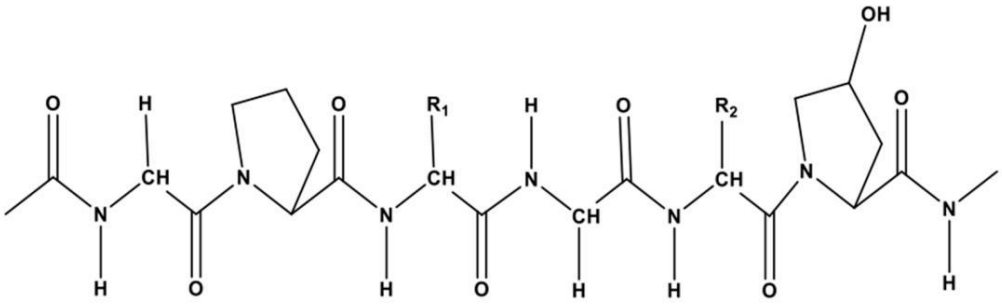

Fig.7: Chemical Structure of Collagen

2.2.3. Properties of collagen

Collagen exhibits a distinct structural characteristic in the form of elongated fibrous arrangements, setting it apart from globular protein enzymes. The process involves catabolic activity driven by collagenolytic enzymes and phagocytosis, which is the outer element of the ECM, forming collagen, which supports cells or tissue and has tensile strength. Collagen is primarily composed of ligaments, muscles, bones, skin, the lens, and the cornea. Findings indicate that collagen fibrils in aged skin display signs of fragmentation and uneven distribution, in contrast to the plentiful, dense, and orderly arrangement of collagen fibrils observed in youthful skin (Shin et al., 2019).

Collagen is a versatile and biodegradable material with desirable features such as biocompatibility, accessibility, versatility, cell compatibility, water affinity, high tensile strength, and body absorbability. Its bioactive properties enable it to create gum or gel-like substances, facilitating cellular healing. Collagen plays a crucial role in wound healing, regulating tissue structure and restoring strength in compromised skin. Skin scarring from burns, surgeries, and physical trauma strains the healthcare system, especially for children with significant scars. Type I and type III collagen are the predominant components of scar tissue (Toshniwal et al., 2019).

TGF-β regulates collagen type I synthesis, promoting scar formation and fibrosis during remodeling. Scar tissue has a unique unidirectional cross-linking pattern, resulting in lower functional quality compared to normal collagen. However, many tissues, like bone, can heal without damaging their structure or function. Collagen has enhanced biocompatibility characteristics and a reduced cytotoxic impact, making it a potential candidate for healing processes. Overall, collagen's unique characteristics make it a promising candidate for repairing and repairing damaged tissues (Agarwal et al., 2016).

Collagen in the human body is adaptable and can be used in bioactive coated platforms to enhance the integration of implantable scaffolds. Hyphapatite (HA) is used for tissue regeneration, and certain fish skin has bioactive properties, making it suitable for human skin treatment. The skin contains eight forms of collagen, each maintaining the skin's smoothness, firmness, and resilience. Enzymatic hydrolysis of triple helix structures generates oligopeptides, which are then converted into bioactive di- and tripeptides. Collagen accumulation on the skin forms a bio-matrix (Bolke et al., 2019).

Being one of the most prevalent structural proteins in the mammalian extracellular matrix (ECM), collagen has unique properties that promote the rapid healing of damaged skin tissue. The primary constituent of connective tissues is collagen type I. Short analogs have also been produced for tissue engineering applications, such as controlling the wound healing response, and they are easily synthesized and purified. The impact of various collagen implants on the promotion of skin and corneal wound healing is covered in this review. These include hydrogels and sponges made of collagen as well as films and membranes (Sklenářová et al., 2022).

Currently, the inclusion of collagen fibers with 50-200 nanometer diameter contributes to the assessment of mechanical properties, owing to their resemblance to protein-like structures. Nevertheless, while considering the perspective of scientists, the mechanical characteristics of collagen remain uncertain. However, it is worth noting that the primary drawback lies in its restricted mechanical properties. The significance of its duty necessitates the utmost importance of its exceptional mechanical qualities (Haaparanta et al., 2014).

Collagen nanofiber mats are being used in scaffolds for organ tissue enhancement due to their exceptional mechanical qualities and flexibility. These biomaterials provide temporary support to damaged organs, enhance cell adhesion and proliferation, and are influenced by factors like material creation, porosity, thickness, and diameter. Synthetic compounds and natural polymers are used to enhance mechanical strength and low wear strength in transplantation procedures. Collagen impacts cellular processes like chemotaxis, adhesion, migration, and morphogenesis, and polymeric aggregations of collagen-platelets contribute to hemostatic effects in human body cells (Golieskardi et al., 2020).

2.2.4. Contribution of collagen in tissue regeneration

The current evaluation of fish-derived extracts as dietary supplements for their ability to facilitate skin regeneration is limited (Lapi et al., 2021). Tissue engineering can be achieved through two distinct methodologies. Two types of biomaterials can facilitate tissue regeneration: biomaterials that only promote tissue regeneration, and biomaterials that incorporate growth factors and supportive cells to further increase tissue regeneration. In the context of skin injury treatment, Cinzia and her research team propose the utilization of sea urchins as a source of native collagen in the development of collagen-based skin-like scaffolds (CBSS). Specifically, they suggest employing sea urchin food waste to construct bilayer CBSS, which consists of both two-dimensional (2D) and three-dimensional (3D) components. The present study describes a methodology centered around the isolation of collagen that is abundant in fibrillar glycosaminoglycan (GAG) from discarded sea urchin gonads (Dong & Lv, 2016).

The characteristics of sea urchin-sourced chondroitin sulfate-based biomaterials were examined, focusing on their microstructure, mechanical stability, water permeability, and ability to prevent bacterial infiltration and promote fibroblast proliferation. Results showed that a 2D collagen membrane effectively mitigates water evaporation and protein diffusion, while three-dimensional collagen scaffolds mimic the dermal layer structure and function, facilitating fibroblast infiltration. These findings suggest potential environmental sustainability and economic feasibility for tissue regeneration (Ferrario et al., 2020).

To generate collagen sponges possessing hemostatic characteristics, the process involves the crosslinking of collagen type I derived from the jellyfish R esculentum using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC). Research conducted on animal subjects has demonstrated that the utilization of sponges composed of EDC collagen exhibits a notable ability to rapidly absorb blood. This can be attributed to their substantial capacity for water absorption, as well as their facilitation of blood cell aggregation at the location of a recent lesion (Cheng et al., 2017). Biomaterials derived from collagen sourced from marine sponges belonging to the *Hexactinellida* class exhibit notable efficacy in the context of bone regeneration. In addition to possessing silicon, calcium, and spongin as constituents of their spicules, these sponges also can undergo regeneration. The primary roles of these structures are to provide structural support for the organism during its growth and development, as well as to provide as a means of protection against potential predators. The chondrogenic properties of collagen isolated from jellyfish, specifically *Rhopilema esculentum*, have been seen to resemble those of human collagen I in animal tests. This suggests that jellyfish collagen could potentially serve as a viable alternative to traditional swine collagen I scaffolds in the field of cartilage regeneration (Felician et al., 2018).

2.3. Hydrogel preparation by different crosslinking methods

Collagen-based hydrogels are created by self-assembling collagen fibers in aqueous solvents. However, their properties decrease during extraction and application, necessitating modification for biological uses. Changes in solvent conditions, external additives, and crosslinking mechanisms can influence the structure and properties of these hydrogels, modulating collagen molecule self-assembly (Cheng et al., 2019).

2.3.1 Solution Condition

Collagen-based hydrogels are subject to the effect of multiple parameters, including solution conditions such as collagen concentration, polymerization temperature, and polymerization pH (Freytes et al., 2008). There exists a causal relationship between the self-assembly of the collagen base and the concentration of collagen (Stepanovska et al., 2021). Studies show a correlation between collagen concentration and hydrogel mechanical properties. An increase in collagen concentration reduces pore size in collagen-based hydrogels, potentially impacting

cell inoculation and viability. The manufacturing process of collagen hydrogels is significantly influenced by temperature. Most gelation occurs at 37°C, but some studies use 25°C or cryogenic conditions. The process can A causal relationship exists also occur under cryogenic conditions, characterized by temperatures below zero degrees Celsius (Carvalho et al., 2022).

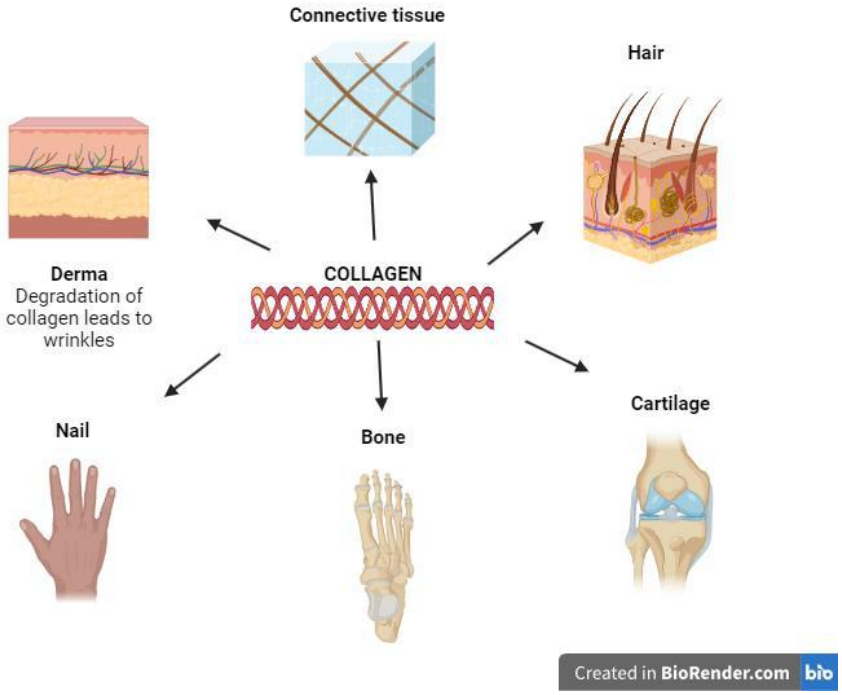

Fig. 8: Role of collagen in tissue regeneration

2.3.2 Physical Crosslinking

Physical crosslinking is a process that creates a three-dimensional network structure of collagen using physical stimuli like UV light, γ-ray irradiation, heating, and freeze-drying. This creates a viscoelastic gel system without introducing harmful chemicals. Dehydrothermal (DHT) treatment is another technique, subjecting collagen molecules to high temperatures in a vacuum environment. However, DHT results in collagen deformation, which can take several days to occur. Ultraviolet radiation can induce unpaired electrons in aromatic amino acid residues, specifically chromic acid and phenylalanine. The irradiation of collagen molecules can result in the generation of ions, which in turn can facilitate the creation of crosslinking between nearby collagen molecules (Bax et al., 2019). Nevertheless, collagen exhibits sensitivity towards UV radiation. Collagen degeneration can occur because of very elevated temperatures and prolonged periods of exposure. The processes of crosslinking and denaturation are in opposition to one other when exposed to UV irradiation. The ultimate equilibrium of these two processes has an impact on the ultimate mechanical characteristics and degradation of collagen biomaterials (Sionkowska et al., 2020).

Crosslinking, a complex process involving physics and chemistry, has garnered significant interest from researchers due to its complex nature and difficulty in precise transformation (L. Xu et al., 2022). Typically, this method entails the interaction between the photosensitizer and ultraviolet (UV) radiation, resulting in the formation of intra- and intermolecular connections inside the collagen fibers. The crosslinking reaction of collagen caused by UV-riboflavin or UV-GelMA is a widely employed method in the field of skin tissue treatment (Yang et al., 2022).

2.3.3 Chemical Crosslinking

Chemical crosslinking is a method used to improve the properties of biomaterials by altering functional groups within collagen. This process results in the formation of a desirable collagen hydrogel by crosslinking polymer chains. Crosslinking facilitates the conversion of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) into urea derivatives that are soluble in water, while ensuring that they are not released into the collagen matrix (Grabska-Zielińska et al., 2022).

EDC/HNS (N-hydroxysuccinimide) shows a reduced level of cytotoxicity, while significantly improving the physicochemical characteristics of collagen (Feng et al., 2020). The collagen scaffold possesses several active groups, including hydroxyl and ester bonds, which have the

capability to directly interact with amino acids or proteins. Dialdehyde starch (DAS), a polymeric aldehyde compound, is synthesized through a chemical interaction between native starch and periodate. It initiates a crosslinking reaction with collagen's amino and imino groups, preserving its fundamental structure and enhancing its biological stability. DAS is characterized by minimal toxicity, biodegradability, antiviral capabilities, and mechanical properties (L. Xu et al., 2022).

2.3.4 Enzymatic Crosslinking

Enzymatic crosslinking is preferred over physical and chemical methods due to its advantages such as mild reaction conditions, absence of byproducts, exceptional specificity, and enhanced catalytic efficiency. It uses specialized enzymes like LOX (lysyl oxidase), MTG (glutamine transaminase), and HRP (horseradish peroxidase) to modify amino groups and produce protofibril connections (Ying et al., 2019). The observed phenomenon demonstrates a notable level of catalytic efficiency and a desirable degree of response to the surrounding environment. In physiological circumstances, the stability of collagen is achieved through enzymatic processes that occur after the translation of its protein sequence. This mechanism facilitates the preservation of collagen's structural integrity, flexibility, and physiological functionality (Zhao et al., 2016). Glutamine transaminase (MTG) has emerged as a prevalent enzyme employed for enhancing the mechanical strength of collagen (Lei et al., 2020).

The enzyme glutamine transaminase is responsible for the conversion of glutamine residues in collagen's γ-hydroxylamine group into an acyl receptor. Subsequently, the acyl transfer process facilitates the formation of an isopeptide bond, leading to the establishment of a covalent cross linkage inside the collagen structure (Jiang et al., 2019). Horseradish peroxidase (HRP) is a plant peroxidase that has been extensively utilized in commercial applications. The enzyme known as horseradish peroxidase (HRP) facilitates the synthesis of polymers rich in phenolic compounds through the utilization of hydrogen peroxide (H_2O_2) as an oxidizing agent (Frayssinet et al., 2020). Collagen comprises several tyrosine residues that can undergo oxidation by the HRP-H_2O_2 system, resulting in the production of active free radicals. These radicals can then engage in polymerization processes with collagen (Cao et al., 2020). Laccase, often known as LAC, is an oxidase that contains multiple copper ions. Evidence for the enhanced stability of collagen by crosslinking with LAC has been noticed (L. Xu et al., 2022).

2.4 Hydrogel preparation via chitosan crosslinking

Chitosan's polysaccharide chains are anchored by hydrogen bonding, hydrophobic interactions, and ionic interactions, as per intermolecular forces. The molecular weight and ionic strength have been shown to exert an influence on these interactions. The process of cross-linking chitosan polymers is imperative for enhancing chitosan's inherent characteristics, particularly its stability and endurance, with the ultimate objective of facilitating drug delivery. The categorization of chitosan-based hydrogel networks is determined by the method employed for chitosan cross-linking and manufacturing (Qun & Ajun, 2006).

2.4.1 Preparation of chitosan hydrogels via chemical cross-linking

Chemically cross-linked hydrogels are produced by covalently linking chitosan macromers, leading to the development of bonds that cannot be reversed. Chemical cross-linked hydrogels can be classified into four unique states of formation, namely: a) chitosan cross-linked system, b) hybrid polymer networks (HPN), c) interpenetrating polymer networks (IPN), and d) semi interpenetrating polymer networks (SIPN).

The phenomenon of chemical cross-linking in chitosan chains can be linked to the presence of amines and hydroxyl groups. Chemical cross-linking can be accomplished through the utilization of cross-linkers or by means of a photopolymerization reaction (Sheng et al., 2019).

2.4.2 Physical cross-linking

Another type of crosslinking involves the use of physical interactions to create chitosan-based hydrogel networks. Ionic interactions can give rise to the formation of ionically cross-linked chitosan hydrogels and polyelectrolyte complexes. Additionally, secondary interactions can occur within networks known as grafted chitosan hydrogels and entangled chitosan hydrogels (Berger et al., 2004).

2.4.3 Chemical versus physical cross-linking

Hydrogel stability depends on the cross-linking mechanism used. Covalent cross-linked hydrogels have resilience against environmental conditions but require additional purification to remove harmful agents. Physically cross-linked hydrogels have higher biocompatibility due to the absence of chemical cross-linkers, making them more bearable. However, these materials may have restricted mechanical stability and are susceptible to environmental changes like pH, temperature, or ionic strength fluctuations. Therefore, it's crucial to incorporate extra purification procedures for these systems (Berger et al., 2004). The unique characteristic of physically cross-linked hydrogels is

highly advantageous in the development of stimuli-responsive systems that exhibit sensitivity to environmental conditions. These systems have the potential to be utilized for targeted medication administration under specific circumstances (Zhang et al., 2009).

III. CHITOSAN AND COLLAGEN COMPOSITE HYDROGELS PREPARATION USING DIVERSE POLYMERS

3.1. Chitosan and collagen based composite hydrogels with natural polymers

Natural polymer hydrogels exhibit a diminished antigenicity, favorable biocompatibility, and a reduced likelihood of eliciting immunological rejection. In addition, some natural polymer hydrogels possess intrinsic antibacterial characteristics, including chitosan and cellulose. Nevertheless, the antimicrobial characteristics exhibited by these natural polymers are insufficient for their application in clinical settings. Previous research has demonstrated the incorporation of antibiotics and antibacterial agents, including metal nanoparticles and conventional drugs, into hydrogels (Zhong et al., 2020). Furthermore, sophisticated techniques have been employed to fabricate diverse multifunctional hydrogels that exhibit notable characteristics such as potent antibacterial activity and controlled release of therapeutic agents under specific conditions (e.g., pH-responsive hydrogels, photo-controlled release hydrogels, thermoresponsive gels) (Trombino et al., 2019). Numerous natural polymers serve as biomaterials in the formulation of hydrogels; however, their utility is hindered by inherent drawbacks such as inadequate stability, mechanical characteristics, and vulnerability to degradation. Synthetic polymers are commonly used in hydrogel wound dressings due to their superior stability, mechanical characteristics, and ability to address mechanical and viscoelastic challenges, making them a popular choice (Raus et al., 2021).

3.1.1. Alginate

Alginate, a marine biopolymer derived from brown seaweed, is widely used in medical dressings due to its biocompatibility, non-toxicity, hydrophilicity, and hemostatic properties (Gunes & Ziylan Albayrak, 2021). Injectable hydrogels are extensively utilized in various applications due to their notable characteristics, including high solubility in aqueous solutions, fast gelation, exceptional flexibility, and biocompatibility (Zhao et al., 2020). A new composite hydrogel was prepared from double net xanthan gum (XG) and dopamine-modified oxidized sodium alginate (OSA-DA), specifically designed for wound dressing and skin simulation sensing. Hydrogel possesses the advantageous characteristics of self-healing and injectability, which allow it to effectively fill wounds of various shapes and sizes, as well as provide a suitable covering for the skin surface to facilitate monitoring. The hydrogel's capacity to function as a sensor establishes a basis for investigating a novel hydrogel dressing that combines biological and sensing functionalities, thereby facilitating human health monitoring. Reactive oxygen species (ROS) in the vicinity of injured tissue can cause potential DNA and protein impairment impede tissue regeneration (Zhao & Yuan, 2022).

It has been observed that cannabinol (CBD) possesses the capacity to impede the production of superoxide free radicals originating from oxidase enzymes NOX1 and NOX4, along with oxidase enzyme XO. Furthermore, it has been observed that CBD exhibits the ability to reduce the production of reactive oxygen species (ROS) (Atalay et al., 2019). Zn^{2+} exhibits antibacterial capabilities across a wide range of bacterial strains. Additionally, it has been found that Zn^{2+} has the ability to modulate the expression of the vascular growth factor (VEGF) gene and facilitate the process of angiogenesis (Atalay et al., 2019). A novel hydrogel composed of zinc alginate (AlgZn) infused with cannabinol (CBD) was prepared. This was achieved by incorporating CBD and Zn2+ ions into a pre-existing sodium alginate hydrogel matrix. The findings from in vitro investigations on hydrogels shown that CBD/Alg-Zn hydrogels had remarkable attributes in terms of anti-inflammatory, antioxidant, and antibacterial effects. Thus, these hydrogels hold promise as innovative wound dressings with multifunctional capabilities for facilitating wound healing (Zheng et al., 2022). Furthermore, the integration of mineral constituents into the polymer matrix has the potential to induce the inherent proliferation of living tissue and facilitate the restoration of compromised anatomical structures. This issue poses significant complexities within the field of regenerative medicine (Ma & Yu, 2021). A PVP-HA-SA hydrogel membrane was developed by adding hydroxyapatite (HA) in-situ. This enhanced adhesion, decreased cytotoxicity, and encouraged tissue growth and wound healing. Experiments showed that higher concentrations of PVP can improve the hydrogel membranes' adhesion properties, mitigating HA-induced toxicity. The membranes exhibit robust adhesion and minimal toxicity, facilitating cell tissue growth and potentially serving as a bandage for wounds on the human body (Fadeeva et al., 2021).

3.1.2. Hyaluronic Acid (HA)

Hyaluronic acid, a natural polymer with biocompatibility, degradability, high hydrophilicity, moisturizing properties, and wound healing capabilities, is

widely used in the biomedical domain. Additionally, it is frequently employed in the fabrication of hydrogel dressings (Alven & Aderibigbe, 2021). The natural deep eutectic solvent (DES) exhibits favorable biocompatibility and has significant efficacy in facilitating the process of wound healing. As a result, it holds considerable promise for utilization within the domain of innovative hydrogel wound dressings (Y. Wang et al., 2020). An innovative antibacterial material called DES-DASH@Ag hydrogel wound dressing, was introduced. This material was prepared by filling a deep eutectic solvent (DES) with lyophilized solute. The DES was then combined with a DASH polymer network, resulting in the formation of the antibacterial material. Glucose and choline chloride exhibit properties that facilitate skin tissue regeneration and mitigate the risk of wound infection (Li et al., 2021). The DES-DASH@Ag hydrogel wound dressing, made of AgNPs synthesized through dopamine reduction and sodium hyaluronate coating, shows significant antibacterial and biocompatibility properties, with cellular activity exceeding 80%. Its sponge-like structure facilitates water absorption and interception, creating a moist environment for wound healing (Qian et al., 2020). Photo crosslinking, a technique used in wound dressings, has shown significant potential in clinical medicine due to its rapid crosslinking rate, minimal invasiveness, and ability to manage the process duration. This advancement in in situ hydrogels is gaining interest (Pang et al., 2021). Nevertheless, the light initiator utilized in the photocrosslinking reaction often exhibits drawbacks such as limited solubility in water and potential harm to living cells. The absence of initiators during UV treatment in the crosslinking reaction of hydrogels has been seen to result in the non-generation of hazardous chemicals. The dual network structure exhibits remarkable mechanical strength and toughness simultaneously, hence endowing the produced hydrogels with a diverse range of exceptional functionalities (Sun et al., 2020). A dual network hydrogel (CMC-AZ/HANB) was developed with various functionalities, composed of azide-functionalized CMC (CMC-AZ) and o-nitrobenzyl modified HA (HA-NB). The hydrogel's mechanical properties were enhanced, and the use of amoxicillin as a medicine within hydrogels showed significant wound healing efficacy (Mao et al., 2022).

3.1.3. Gelatin

The low mechanical strength of chitosan can be efficiently addressed by blending its biopolymer with other polymers (Hong et al., 2019). A covalently antibacterial alginate-chitosan hydrogel dressing that incorporated gelatin microspheres loaded with tetracycline hydrochloride was developed. This was achieved through the utilization of the emulsion crosslinking approach, with the purpose of enhancing wound healing. The microspheres containing antibiotics are integrated into the gel scaffolds, resulting in both sustained drug release and improved mechanical qualities. The principal objective was to augment the mechanical characteristics and drug administration capacities of hydrogels obtained from natural sources (H. Chen et al., 2017).

The application of antibacterial composite hydrogel dressings has been shown to effectively enhance wound healing by addressing and treating infections. To provide a visual representation, the experiment demonstrated the phenomenon of growth inhibition in relation to *Escherichia coli* and *Staphylococcus aureus* (Sudheesh Kumar et al., 2012). Novel wound dressing materials were developed using gelatin and gelatin-based chitosan bilayers. Gelatin's biocompatibility and biodegradability make it ideal for medicine and pharmaceuticals. The study used an ex vivo model to investigate biocompatibility. The cross-linked gelatin provides mechanical support to hydro-films, while a porous structural matrix shows swelling capacity. No cytotoxicity was found during in vitro testing. The bilayer hydrofilm in human skin showed satisfactory biocompatibility, indicating its potential for wound healing (Garcia-Orue et al., 2019).

A multifunctional hydrogel, the Gel/TA (Gelatin-Tannic Acid) hydrogel, was synthesized using gelatin and tannic group functional groups. This hydrogel has the potential to be used in full-thickness wound management and release active compounds within wound settings. The hydrogel's cytocompatible properties, ability to induce ECM regeneration, and promotion of cell adhesion and proliferation have been shown to improve wound healing processes. The gel-based composite hydrogel has potential for clinical management of full-thickness wounds (Ahmadian et al., 2021).

The antioxidative action of the Gel/TA hydrogel is attributed to TA, which possesses a polyphenolic structure and contains many hydroxyl (OH) groups (Jimoh et al., 2016). Nevertheless, research indicates that the process of gelation in hydrogel formation involves the occurrence of hydrogen bonding cross-linking (Kamoun et al., 2017). In contrast, the impact of adding 2-hydroxyethyl methacrylate (HEMA) and poly (ethylene glycol) methyl ether methacrylate (PEGMA) to Gel-N-acetylcysteine hydrogels was studied. These polymers enhance wound healing and blood clotting abilities. The antioxidant activity of these hydrogels is attributed to the conversion of amino groups into amides through dehydrothermal cross-linking treatment. The study also found a decline in antioxidant cross-linking in hydrogels derived from N-acetylcysteine

due to decreased amino group accessibility. This suggests that dehydrothermal crosslinking significantly enhances the antioxidation ability of gel-based hydrogels (Gomez-Aparicio et al., 2021).

3.1.4. Agarose

Agarose, a linear polysaccharide, is widely used in biological investigations due to its minimal interaction with biomolecules, robust properties, and ability to form gels. Its high biocompatibility makes it an attractive biomaterial for tissue engineering and medicine delivery, as tissue regeneration relies on biocompatibility and biodegradability. Its application in electrophoresis and bacterial cell culture is also significant (Yamada et al., 2020). Agarose-based hydrogels show potential in regenerative medicine, especially in producing human skin and organs. Agarose, an injectable polymer, is gaining attention due to its in-situ polymerization, reduced invasiveness, on-site shape ability, and potential for targeted cell and signaling molecule delivery (Irastorza-Lorenzo et al., 2021). The utilization of agarose scaffolds has demonstrated enhanced surgical outcomes and improved consistency in cell phenotypic. Although agarose is widely recognized as a highly effective medium for facilitating tissue regeneration, there has been limited investigation into its application inside three-dimensional tumor models (Salati et al., 2020).

The thermal crosslinking method is the predominant technique employed for the manufacture of agarose scaffolds. This approach entails employing the gelation procedure, which the utilization of microwave radiation can execute. A thermal crosslinking technique was used to fabricate an agarose scaffold. The process involved dissolving agarose (1.5% weight) in aqueous solvent using microwave heating, with temperatures ranging from 60 ◦C to ambient temperature. The cooling rate was set at 30˚C/min. The blending of agarose with other polymers resulted in an augmentation of cellular activity and cell regeneration (Varoni et al., 2012).

A composite material combining agarose and silk for cartilage regeneration was developed. The presence of the silk/agarose scaffold also increased the expression of cartilage-specific marker genes, such as aggrecan, sox-9, and collagen Type II, which improved the agarose microenvironment for chondrocyte culture (Singh et al., 2016).

CHO-agarose hydrogels and peptide-agarose microgel scaffolds were developed using oxidizing 2,2,6,6–(tetramethylpiperidin–1– yl)oxyl. The peptide-agarose microgel scaffold-based 3D cell culture system demonstrated its efficacy as a biomaterial for tissue engineering, enhancing cell proliferation in a three-dimensional environment (Yamada et al., 2020). A nanocomposite of agarose-gelatin-glass nanoparticles was developed using freeze gelation. The compound was dissolved in distilled water, then glass nanoparticles were added to the gelatin mixture. Lyophilization allowed for the formation of a scaffold, which could be used in treating osteomyelitis by enhancing the hydroxyapatite layer in bodily fluids, thereby promoting tissue regeneration. The scaffolds have potential applications in various medical applications (Ali et al., 2021). The utilization of agarose-based scaffolds offers several notable advantages, including robust physical crosslinking, responsiveness to changes in temperature, and enhanced stability even at lower concentrations (Utech & Boccaccini, 2016).

3.1.5. Dextran

Dextran, an exopolysaccharide, is produced by lactic acid bacteria using sucrose as a primary substrate, consisting of a linear chain of D-glucopyranose units linked by α (1➞6) bonds. Additionally, there are varying numbers of branching connections, such as α–(1➞2), α–(1➞3), and α–(1➞4), present within the molecule. The molecular weights of these molecules typically range around 40 kDa (Díaz-Montes, 2021). The compound possesses chemically reactive hydroxyl groups, which enable its modification with various functional groups, resulting in the formation of spherical, tubular, and three-dimensional networks. Dextran-based scaffolds that are biodegradable have the ability to serve as carriers for bioactive protein biomolecules, enabling controlled release and facilitating tissue regeneration. Dextran hydrogels have the potential to serve as a matrix for bioartificial cardiac tissue (BCT) in in-vitro regeneration. These hydrogels offer unique benefits for soft tissue engineering due to their resistance to protein adsorption and cell adhesion, enabling the creation of targeted recognition sites (Banerjee et al., 2021). The creation of a crosslinked network of dextran hydrogels through radical methacrylate group polymerization, resulting in macroporous scaffolds with a beaded-wall morphology due to the lack of miscibility between the dextran matrix and poly(ethylene glycol). These scaffolds demonstrated the ability to effectively handle liquid-liquid phase separations and exhibited enhanced cell penetration and nutrient diffusion capabilities (Lévesque et al., 2005). These carriers could serve as bioactive agents for various protein biomolecules that possess inherent biodegradability. However, it has been noted that these carriers are more expensive and exhibit lower bioavailability (Varghese et al., 2020).

3.2. Chitosan and collagen-based composite hydrogels with synthetic polymers

Numerous natural polymers serve as biomaterials in the formulation of hydrogels; however, their utility is hindered by inherent drawbacks such as inadequate stability, mechanical characteristics, and vulnerability to degradation. On the other hand, synthetic polymers exhibit enhanced stability, superior mechanical characteristics, and the ability to manipulate their structure and properties, and they effectively address the mechanical and viscoelastic challenges associated with hydrogels. Consequently, hydrogel wound dressings frequently incorporate them in their formulation (Raus et al., 2021).

3.2.1. Polyvinyl alcohol (PVA)

PVA's superior water solubility, biocompatibility, biodegradability, non-carcinogenicity, mechanical qualities, and ease of processing have led to substantial research on the material's potential for wound healing (Su et al., 2021). Nevertheless, the elasticity of PVA hydrogels is insufficient, and their hydrophilicity is also limited, hence constraining their standalone application as polymer materials for wound dressings. The utilization of intricate combinations comprising both natural and synthetic polymers is significant in enhancing the mechanical and physicochemical characteristics of PVA hydrogel dressings. Hence, several composite polymers have been employed in the fabrication of wound dressings based on polyvinyl alcohol (PVA). The careful selection and manufacturing techniques of these composites are of utmost significance, as the characteristics of PVA hydrogels are contingent upon the properties of the incorporated ingredients (Radulescu et al., 2022).

Polyvinyl alcohol (PVA) is a promising material for hydrogel dressings due to its bioactivity, which prevents cell growth. Composite materials like chitosan and alginate can increase PVA's bioactivity. These dressings have shown promise in medication delivery systems and wound dressings, expediting wound healing by releasing therapeutic agents like medicines, DNA, growth factors, nanoparticles, and proteins. An experimental study aimed to develop PVA hydrogels with zinc oxide nanoparticles and mesoporous silica nanoparticles to create effective treatments for infected wounds using cephalexin antibiotics (Nikdel et al., 2021). Furthermore, there is a growing body of research exploring the utilization of electrospinning as an alternative manufacturing method to produce nanofiber hydrogels, specifically for the delivery of wound-healing active materials. This approach aims to replace conventional manufacturing methods now in use. Propolis nanoparticles are being utilized in wound healing and tissue regeneration by being incorporated into wound dressings composed of cross-linked polyvinyl alcohol (PVA) and propolis polymer nanofibers (Alberti et al., 2020).

3.2.2. Poly (N-isopropylacrylamide)

Poly (N-isopropylacrylamide) (PNIPAM) is a thermos reversible hydrogel with a low critical solution temperature (LCST) of 32°C. Its cross-linked gels exhibit expansion and contraction phenomena, allowing continuous medication delivery based on physiological signals. PNIPAM has been extensively researched as a controlled drug delivery system (Sun et al., 2019). Thermosensitive polyvinyl alcohol (PVA) hydrogels were fabricated by using SA-g-N-isopropyl acrylamide, a thermosensitive copolymer, for wound management. The polymer was synthesized using redox co-polymerization, achieving a drug release rate of 65% at ambient temperature and 35% at temperatures above the human body's normal range (Montaser et al., 2019).

3.2.3. Polyethylene Glycol (PEG)

Polyethylene glycol (PEG) is a water-soluble, amphiphilic polyether with both hydrophilic and hydrophobic properties. As a transparent, colorless liquid, PEG is highly compatible with biological systems and exhibits exceptional biocompatibility and biodegradability. A bilayer dressing was developed using PVA-CMC-PEG hydrogels using a freeze-thaw technique. They manipulated the hydrogel's pore size, resulting in a thorough combination of two layers. The double-layer dressing had favorable mechanical qualities, impeding germ infiltration, and regulating moisture loss from the wound. This created a moist environment leading to enhancing wound healing (Y. Li et al., 2019). A semi-interpenetrating hydrogel dressing was developed using PEG diacrylate, PVA, and gum tragacanth. The addition of PVA increased swelling rate and reduced porosity. Increased PEG diacrylate increased cell adhesion and network elongation. Reduction in PEG diacrylate reduced network disintegration. The dressing is non-toxic and has antibacterial efficacy (Hemmatgir et al., 2022).

3.2.4. Poly (lactic-co-glycolic acid)

Poly (lactic-co-glycolic acid) (PLGA) is a FDA-approved synthetic polymer widely used in pharmaceutics and tissue engineering due to its biocompatibility, adjustable mechanical properties, and manageable degradation rate. It can be easily manufactured by adjusting the copolymer ratio between lactic acid and glycolic acid (Sezlev Bilecen et al., 2019). Periodontal tissue regeneration requires materials that meet the necessary requirements. Synthetic polymers have favorable mechanical and biological attributes but lack biocompatibility. Natural polymers have hydrophilicity and biocompatibility but lack the necessary mechanical features. Research groups are developing periodontal materials for guided tissue regeneration (GTR) and guided

bone regeneration (GBR) by combining natural polymers with inorganic or synthetic polymers to achieve the necessary characteristics (Forero et al., 2017). The combination of PLGA and CS can be readily achieved using diverse methodologies to form nanoparticles. The utilization of PLGA nanoparticles (nPLGA) and CS nanoparticles (nCS) as drug delivery systems has been widely employed in many applications. Nano-sized materials are commonly preferred in periodontal surgery, making it necessary to synthesize nanoparticles. This study aims to synthesize periodontal tissue regeneration materials by combining nPLGA, nCS, and nAg. The objective is to develop a biocompatible substance that promotes cellular mineralization and reduces periodontal disease relapse. The research involved synthesizing nanoparticles of PLGA, CS, and Ag, and investigating their cytotoxicity and impact on cell calcification. After understanding the characteristics of the materials, they were mixed and evaluated for their cytological properties. This is the first research on this combination (Ma et al., 2018).

IV. EFFECT OF TEMPERATURE AND pH ON CHITOSAN-COLLAGEN HYDROGELS

The effects of temperature on hydrogels can either be positive or negative. If gel material swells with the rise in temperature and pH then it is a positive response. If shrinks, then it is a negative response (Huang et al., 2019).

4.1. Temperature-sensitive nature of hydrogels

Temperature-sensitive hydrogels can be categorized into two types based on their structure: negatively thermo-sensitive hydrogels and positively thermo-sensitive hydrogels. Polymers having LCST can negatively generate temperature sensitive hydrogels, the polymers shrink along with the temperature increases. At reduced temperatures, the prevalence of hydrogen bonding between hydrophilic groups within the polymer chain and water molecules becomes prominent, resulting in the solubility of the polymer in water. Nevertheless, as the temperature rises, the hydrophobic contacts between hydrophobic groups are enhanced, whilst the strength of hydrogen bonding diminishes, resulting in the phenomenon of gelation. Hydrogels that exhibit a positive temperature sensitivity demonstrate an increase in their solubility in water as the temperature rises, hence exhibiting an upper critical solution temperature (UCST) (Huang et al., 2019).

4.2. pH-sensitive behavior of hydrogels

pH-responsive polymers (PRPs) exhibit a capacity to undergo structural and property modifications in response to fluctuations in environmental pH. These alterations encompass changes in surface activity, chain structure, conformation, solubility, and configuration. pH-sensitive polymers are characterized by the presence of acidic or basic functional groups, which exhibit the ability to either take or donate protons in response to changes in the surrounding pH conditions. PRP types are present and can be differentiated based on categorization criteria, including distinguishing weak bases from weak acids, as well as differentiating biopolymers and degradable polymers from synthetic polymers. Nanoparticles are utilized in several fields, such as medicine delivery, gene delivery, actuation and sensing/biosensing, and separation techniques, among others (Wei et al., 2017).

Chitosan demonstrates pH-sensitive characteristics as a weak polybase owing to the abundant presence of amino groups along its molecular chain. Chitosan has great solubility in acidic conditions; however, it retains its insoluble nature when exposed to alkaline pH levels. The process of pH-sensitive swelling is initiated by the protonation of the amine groups when exposed to low-pH circumstances. Protonation induces chain repulsion, resulting in the diffusion of protons, counter ions, and water inside the gel matrix, as well as the dissociation of secondary connections. The phenomenon of pH sensitivity has been documented in the context of drug delivery applications (Woraphatphadung et al., 2018). Successful production of pH-sensitive nanocomposites composed of chitosan and silica was documented, with the incorporation of curcumin. The researchers exhibited a release of curcumin that was dependent on pH. The researchers reached a conclusion regarding the adjustable release profile of the active chemical by utilizing the pH of the release medium (Gaware et al., 2019). Other researchers have also demonstrated the pH-dependent release of drugs utilizing polymeric polymers based on chitosan (Ata et al., 2020). A novel chitosan-based film was developed that exhibits pH-dependent color-changing properties, thereby demonstrating its potential as a creative application. Alizarin was integrated into the formulation for the purpose of enhancing the functionality and intelligence of food packaging materials. The composite film exhibited a discernible alteration in color, transitioning from a faint yellow hue to a vibrant purple shade, in response to fluctuations in pH within the range of 4 to 10. The film composed of chitosan and alizarin exhibited antibacterial, antioxidant, and color-changing characteristics in response to changes in pH (Ezati & Rhim, 2020).

4.3. Advantages of temperature and pH-sensitive hydrogels in wound care

Temperature-sensitive hydrogels have demonstrated exceptional potential as carriers for the delivery of diverse biotherapeutic compounds, offering

several notable advantages: (i) Temperature-sensitive hydrogels have the ability to undergo gelation at physiological temperatures, making them more practical for administration purposes. (ii) The hydrogel matrix that is formed can provide protection for delicate drugs and cells that are incorporated within it. (iii) The controlled release of biotherapeutic molecules from hydrogels allows for enhanced efficacy and a reduction in potential side effects. The obstacles and constraints associated with the use of temperature-sensitive hydrogels as delivery vehicles for biotherapeutic compounds include the mechanical strength of the swelling gel, its low temperature sensitivity, and the biocompatibility of the polymers (Huang et al., 2019).

4.3.1 Effect of temperature and pH sensitivity of chitosan-collagen hydrogels

Thermo and pH-responsive hydrogels were developed and loaded with doxorubicin (DOX) with potential therapy of breast cancer. Swelling studies of hydrogels and their morphology implied the porous structure, high water content with rapid swelling/deswelling rate in response to abrupt changes of pH and temperature. The release investigation of DOX at different concentration, temperature and pH values confirmed the accelerated release of DOX in lower concentration and acidic condition at 37 °C as compared to neutral pH and the temperature of 40 °C.

The proliferation of MCF-7 cells on the prepared hydrogel and DOX-loaded hydrogel was evaluated by 4′,6-diamidino-2-phenylindole (DAPI) staining which further demonstrated the potential of developed hydrogels for local therapy of breast cancer (Fathi et al., 2019).

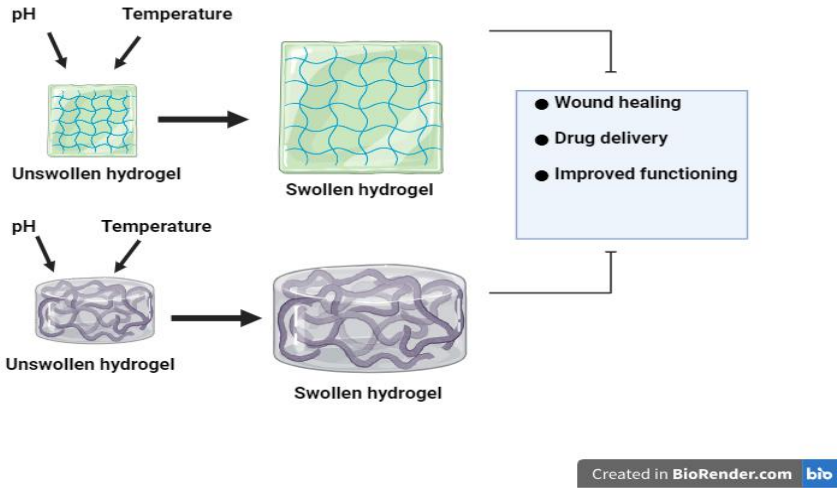

Fig: 9. Effect of temperature and pH on hydrogels

Injectable hydrogels have shown great potential in cell therapy and drug delivery. They can easily fill in any irregular-shaped defects and remain in desired positions after implantation using minimally invasive strategies. Here, we developed hydrogels prepared from tilapia skin collagen and chitosan (HCC). The residual mass rate of HCC was affected by the pH at the time of preparation, which was 29.1 % at pH 7 in 36 h. By comparison, the residual mass ratios of HCC at pH values of 6 and 5 were only approximately 8.4 % and 0, respectively. In addition, the stability of HCC was also affected by the concentration of these two components. HCC10 catalyzed by 10 mg mL^{-1} tilapia skin collagen and 10 mg mL^{-1} chitosan was more stable than HCC5 catalyzed by 5 mg mL^{-1} tilapia skin collagen and 10 mg mL^{-1} chitosan; therefore, we studied the ability of HCC10 to deliver two model nanobodies: 2D5 and KPU. As the concentration of nanobodies increased, the cumulative release rate of 2D5 decreased, and the release rate of KPU increased. Meanwhile, the cumulative release rate of 2D5 was the highest (68.3 %) at pH 5.5, followed by pH 6.8 (56.4 %) and 7.4 (28.4 %). However, the cumulative release rates of KPU were similar at pH 5.5 (45.1 %), 6.8 (46.5 %), and 7.4 (44.9 %). HCC is biodegradable and can facilitate the release nanobodies; thus, HCC could be developed into an intelligent responsive tumor treatment matrix for use in cancer therapy (Maturavongsadit et al., 2020).

Dapsone (DAP) is a bactericidal agent used in the treatment of leprosy caused by *Mycobacterium leprae*. Despite its therapeutic potential, DAP has low solubility,

which results in allow therapeutic index and a high microbial resistance. Recently, new approaches were used to increase the DAP solubility. In particular, the use of interpenetrating polymer network (IPN)-hydrogels based chitosan (CS) for the controlled release of DAP provides some advantages because they can modify their swelling properties and network structures as a response to environmental stimuli. The aim of this study was to synthesize and physiochemically characterize pH-responsive chitosan/polymer hydrogels to control the release of DAP. For this reason, different combinations of polymers, such as polyvinyl pyrrolidone, polyethylene glycol and hydroxypropyl methylcellulose, and concentrations of the cross-linking agents (glutaraldehyde) were used and then blended to the CS. The resulting hydrogels were evaluated in terms of physicochemical and swelling properties, rheological analysis, and in vitro release of DAP at different pHs (1.2–6.8). Hydrogels were further characterized by Fourier transformed infrared (FT-IR) spectroscopy and scanning electron microscopy (SEM) analysis. pH-responsive DAP-loaded hydrogels may represent the set-up for developing potential oral formulations for the treatment of leprosy caused by Mycobacterium leprae (Chaves et al., 2019).

Fucoidans, sulfated polysaccharides from brown algae, possess multiple bioactivities regarding osteogenesis, angiogenesis, and inflammation, all representing key molecular processes for successful bone regeneration. To utilize fucoidans in regenerative medicine, a delivery system is needed that temporarily immobilizes the polysaccharide at the injured site. Hydrogels have become increasingly interesting biomaterials for the support of bone regeneration. Their structural resemblance with the extracellular matrix, their flexible shape, and capacity to deliver bioactive compounds or stem cells into the affected tissue make them promising materials for the support of healing processes. Injectable hydrogels stand out due to their minimal invasive application. In the current study, we developed an injectable thermosensitive hydrogel for the delivery of fucoidan based on chitosan, collagen, and β-glycerophosphate (β-GP). Physicochemical parameters such as gelation time, gelation temperature, swelling capacity, pH, and internal microstructure were studied. Further, human bone-derived mesenchymal stem cells (MSC) and human outgrowth endothelial cells (OEC) were cultured on top (2D) or inside the hydrogels (3D) to assess the biocompatibility. We found that the sol-gel transition occurred after approximately 1 min at 37 °C. Fucoidan integration into the hydrogel had no or only a minor impact on the mentioned physicochemical parameters compared to hydrogels which did not contain fucoidan. Release assays showed that 60% and 80% of the fucoidan was released from the hydrogel after two and six days, respectively. The hydrogel was biocompatible with MSC and OEC with a limitation for OEC encapsulation. This study demonstrates the potential of thermosensitive chitosan-collagen hydrogels as a delivery system for fucoidan and MSC for the use in regenerative medicine (Ohmes et al., 2022).

Mandible defects are a difficult issue in dental surgery owing to limited therapeutic options. Recombinant human bone morphogenetic protein-2 (rhBMP2) is osteoinductive in bone regeneration. This article prepared chitosan/collagen hydrogels with rhBMP2-incorporated gelatin microsphere (GMs) for a sustained release of rhBMP2 to induce bone regeneration in rabbits. The rhBMP2 release profiles in vitro were investigated within a period of 4 weeks. The test groups were hydrogels+10 μg rhBMP2, GMs+10 μg rhBMP2, and hydrogels/GMs+10 μg rhBMP2. These delivery systems were suspended in 2 mL of PBS (pH 7.4) and incubated at 37°C with shaking at 80 rpm for 4 weeks. At predetermined time points, 50 μL of supernatants were harvested and then 50 μL of fresh PBS (pH 7.4) was supplemented. The concentrations of released rhBMP2 in the harvests were detected using the rhBMP2 ELISA kit (R&D Systems, Shanghai, China). The cumulative release of rhBMP2 was computed. Chitosan/collagen hydrogels with rhBMP2- incorporated GMs exhibited an ideal releasing profile of rhBMP2 in vitro. These composite scaffolds had a better capacity to heal mandible defects than the other two hydrogel scaffolds. Chitosan/collagen hydrogels with rhBMP2-incorporated GMs might be potential carriers of rhBMP2 for accelerating the repair of bone defect (Song et al., 2016).

This introduces a novel type of injectable temperature-sensitive chitosan/glycerophosphate/collagen (C/GP/Co) hydrogel that possesses great biocompatibility for the culture of adipose tissue-derived stem cells. The C/GP/Co hydrogel is prepared by mixing 2.2% (v/v) chitosan with 50% (w/w) β-glycerophosphate at different proportions and afterwards adding 2 mg/ml of collagen. The gelation time of the prepared solution at 37°C was found to be around 12 min. The inner structure of the hydrogel presented a porous spongy structure, as observed by scanning electron microscopy. Moreover, the osmolality of the medium in contact with the hydrogel was in the range of 310–330 mmol kg−1. These analyses have shown that the C/GP/Co hydrogels are structurally feasible for cell culture, while their biocompatibility was further examined. Human adipose tissue-derived stem cells (ADSCs) were seeded into the developed C/GP and C/GP/Co hydrogels (The ratios of C/GP and C/GP/Co were 5:1 and 5:1:6, respectively), and the cellular growth was periodically observed under an inverted microscope. The proliferation of ADSCs was detected using cck-8 kits, while cell apoptosis was

determined by a Live/Dead Viability/Cytotoxicity kit. After 7 days of culture, cells within the C/GP/Co hydrogels displayed a typical adherent cell morphology and good proliferation with very high cellular viability. It was thus demonstrated that the novel C/GP/Co hydrogel herein described possess excellent cellular compatibility, representing a new alternative as a scaffold for tissue engineering, with the added advantage of being a gel at the body's temperature that turns liquid at room temperature (Song et al., 2010).

Thermo and pH responsive chitosan–collagen (CHT–CLG) scaffolds were prepared using a non-residue strategy. CHT–CLG scaffolds (pH sensitive) were produced by freeze dryingmethod, cross-linked with glutaraldehyde, and coated with poly (N,N -diethylacrylamide) (PDEAAm) in supercritical media to confer the thermoresponsive behavior. This green and integrated process generated a wide range of porous structures with different mechanical properties, reversible swelling ability and controlled biodegradability, depending on the scaffold composition and cross-linking degree. The ability of these dual sensitive structures to control the release of a low molecular weight drug (ibuprofen, Ibu) and a model protein (BSA) was investigated. Small portions of the native and PDEAAm coated scaffolds (around 20 mg) were placed inside a 50 mL of buffer solutions at different pH (5.5 or 7.4) and temperatures (20 and 37 ◦C). 1 mL aliquots were withdrawn periodically from the solutions and collected in eppendorfs. The release medium was refreshed with buffer solution (1 mL) after sampling. In order to determine the ibuprofen and BSA released, the samples were analyzed in a Helius Alpha DoubleBeam UV/VIS spectrophotometer at 265 and 280 nm, respectively. In order to design a completely clean strategy to develop potential pH and thermo ON–OFF devices with controlled morphological and mechanical properties for drug delivery, chitosan and collagen-based scaffolds were prepared by freeze drying method and coated with PDEAAm in scCO2. By varying the scaffold composition as well as the cross-linking degree, it was possible to tune the morphological and mechanical properties of the blended matrices. The efficiency of the hydrogel coating was evaluated by investigating the mean pore size of the matrices, the swelling–deswelling ability, the biodegradability, cytotoxicity, and mechanical behavior of the PDEAAm-coated scaffolds. Owing to the promising morphological, biological, and mechanical features, the native scaffolds also proved to have a defined pH-sensitive behavior, and the PDEAAm-coated ones showed an effective dual (pH and thermo) sensitive conduct. Moreover, the BSA release profile was revealed to be significantly dependent on pH and temperature effects (higher release profiles at pH 7.4 and 20 ◦C), while the Ibu release behavior demonstrated to be mainly reliant on morphological and mechanical properties of the scaffolds (higher release profiles using CLG scaffolds cross-linked with 1% of GA). Herein it was developed an integrated sustainable strategy comprising scaffold preparation, drug loading, cross-linking process and coating in scCO2 to produce dual thermo and pH responsive chitoswas an–collagen scaffolds for sustained drug delivery; future work will extend the strategy to generate highly regulated dual sensitive porous networks able to release therapeutic antibodies, growth factors, genes and enzymes which are also essential biomolecules for tissue regeneration (Barroso et al., 2014).

In situ 3D printing technologies is a new frontier for highly personalized medicine, which requires suitable bioink with rheology, biocompatibility, and gelation kinetics to support the right shape and mechanical properties of the printed construct. To this end, a facile design of thermo/photo dual cure composite hydrogel was proposed using MHBC and soluble collagen in this study. M/C composite hydrogel exhibited rapid thermo-induced sol-gel transition and contraction, tunable mechanical properties, proper microstructure, and biodegradability for 3D cell culture, as well as improve cyto-compatibility, all of which were dependent upon the methacrylation degree of MHBC and M/C ratios. The printability of the optimal formulation (3% MHBC/1% collagen) was validated by its mild printing condition, rapid gelation of bioink at 37 °C and simple postprocessing manipulation. Both desirable printability and cyto-compatibility enable M/C composite hydrogel, a potential candidate as bioink to be applied for in situ 3D bioprinting (Liu et al., 2021).

Chitosan and collagen are natural biomaterials that have been used extensively in tissue engineering, both separately and as composite materials. Most methods to fabricate chitosan/collagen composites use freeze drying and chemical crosslinking to create stable porous scaffolds, which subsequently can be seeded with cells. In this study, we directly embedded human bone marrow stem cells (hBMSC) in chitosan/collagen materials by initiating gelation using b-glycerophosphate at physiological temperature (37˚C) and pH (7.3-7.4). We further examined the use of glyoxal, a dialdehyde with relatively low toxicity, to crosslink these materials and characterized the resulting changes in matrix and cell properties. The cytocompatibility of glyoxal and the crosslinked gels were investigated in terms of hBMSC metabolic activity, viability, proliferation, and osteogenic differentiation. These studies revealed that glyoxal was cytocompatible at concentrations below about 1 mM for periods of exposure up to 15 h, though the degree of cell spreading, and proliferation were dependent on matrix composition. Glyoxal-crosslinked matrices were

stiffer and compacted less than uncrosslinked controls. It was further demonstrated that hBMSC can attach and proliferate in three-dimensional matrices composed of 50/50 chitosan/collagen, and that these materials supported osteogenic differentiation in response to stimulation. Such glyoxal-crosslinked chitosan/collagen composite materials may find utility as cell delivery vehicles for enhancing the repair of bone defects (Wang & Stegemann, 2011).

Chitosan and collagen type I are naturally derived materials used as cell carriers because of their ability to mimic the extracellular environment and direct cell function. In this study beta-glycerophosphate (b-GP), an osteogenic medium supplement and a weak base, was used to simultaneously initiate gelation of pure chitosan, pure collagen, and chitosan-collagen composite materials at physiological pH and temperature. Adult human bone marrow-derived stem cells (hBMSC) encapsulated in such hydrogels at chitosan/collagen ratios of 100/0, 65/35, 25/75, and 0/100 wt% exhibited high viability at day 1 after encapsulation, but DNA content dropped by about half over 12 days in pure chitosan materials while it increased twofold in materials containing collagen. Collagen-containing materials compacted more strongly and were significantly stiffer than pure chitosan gels. In monolayer culture, exposure of hBMSC to b-GP resulted in decreased cell metabolic activity that varied with concentration and exposure time but washing effectively removed excess b-GP from hydrogels. The presence of chitosan in materials resulted in higher expression of osterix and bone sialoprotein genes in medium with and without osteogenic supplements. Chitosan also increased alkaline phosphatase activity and calcium deposition in osteogenic medium. Chitosan–collagen composite materials have potential as matrices for cell encapsulation and delivery or as in situ gel-forming materials for tissue repair (Wang & Stegemann, 2010).

V. CHITOSAN-COLLAGEN HYDROGELS ANTI-BACTERIAL AND WOUND-HEALING PROPERTIES

The conjugation of chitosan biopolymer with other polymers might successfully address the difficulty of chitosan's low mechanical strength (Garnica-Palafox & Sánchez-Arévalo, 2016). Using an emulsion crosslinking approach, a hydrogel dressing was prepared with antibacterial properties through covalent bonding and gelatin microspheres that transport tetracycline hydrochloride, aiding wound healing. These microspheres, infused with antibiotics, are inserted into gel scaffolds, ensuring continuous drug release, and enhancing mechanical properties. The study aimed to improve the mechanical and drug transport characteristics of natural hydrogels, enhancing wound healing, and addressing infections. The dressings effectively inhibit the growth of *E. coli* and *S. aureus*, which often multiply after infections (H. Chen et al., 2017). Examples of the antibacterial activity of chitosan-based hydrogels are shown in **Table 1**.

Table 1: Different types of chitosan-based hydrogels for antibacterial activity.

Bacterial Species	Hydrogel Type	References
E. coli	PEG–Chitosan Hydrogel	(Sharma et al., 2019)
E. coli and S. aureus	Chitosan/Alginate Hydrogel Dressing Loaded FGF/VE-Cadherin	(Wei et al., 2022)
E. coli, S. aureus and P. aeruginosa	PVA/Starch/Chitosan Hydrogel Membranes with Nano Zinc oxide	(Baghaie et al., 2017)
E. coli and S. aureus	Polyvinyl alcohol (PVA)/N–succinyl chitosan (NSCS)/lincomycin hydrogels	(Qing et al., 2021)
S. aureus	Chitosan/PVA-Based Hydrogel Films	(Mohite et al., 2023)

Innovative wound dressing materials were developed by combining gelatin and chitosan in a bilayer structure. Gelatin is widely used in medical and pharmaceutical applications due to its biocompatibility and biodegradability **(Fig. 10).** The materials can be used for wound healing due to their mechanical reinforcement and swelling capacity. The porous structural matrix showed no in vitro cytotoxicity, ensuring the hydro-film's mechanical integrity. The bilayer hydrofilm in human skin showed significant biocompatibility (Garcia-Orue et al., 2019). Researchers are now exploring hydrogen-bonded extracellular matrix-mimicking hydrogels for wound treatment. A hydrogel was made mimicking the extracellular matrix (ECM) using gelatin and tannic groups. The Gel/TA hydrogel is suitable for full-thickness wound treatment, releasing active chemicals and promoting wound healing through cytocompatibility, regeneration, and cell adhesion. The antioxidation action of the Gel/TA hydrogel

is attributed to the polyphenolic structure of TA and the abundance of OH groups it contains (Ahmadian et al., 2021). Investigations have shown that hydrogen bonding cross-linking takes place throughout the process of hydrogel formation, leading to gelation (Kamoun et al., 2017). Gel-N-acetylcysteine hydrogels were enhanced with HEMA and PEGMA, enhancing wound healing and blood clotting. The antioxidant activity decreases with dehydrothermal cross-linking, affecting the antioxidation capabilities more than uncross-linked polymers (Gomez-Aparicio et al., 2021).

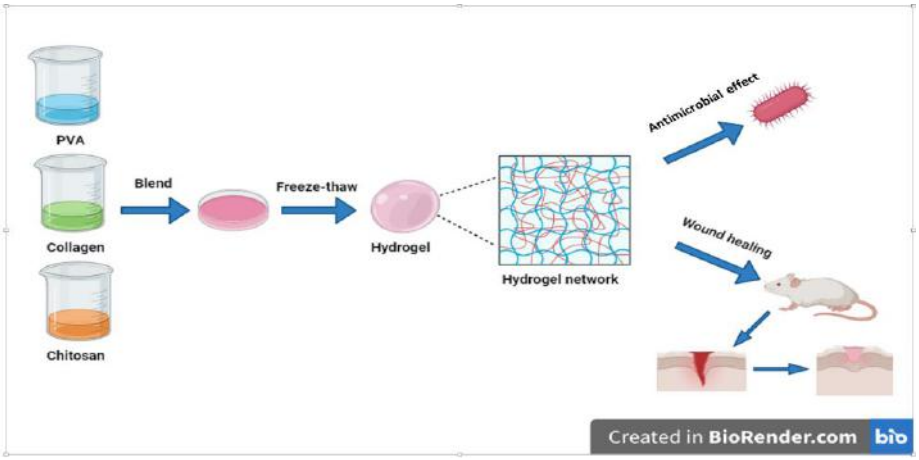

Fig.10: Chitosan-collagen have antimicrobial and wound healing effects

Skin diseases are a significant and critical clinical concern. Hydrogels, which mimic the human body's extracellular matrix, can be used as regenerative scaffolds to address skin defects. Synthesized by blending hyaluronic acid (HA) and carboxylated chitosan (CCS) with human-like collagen (HLC), these hydrogels have been tested for their biocompatibility. The hydrogels significantly enhanced the adhesion, proliferation, and migration of L929 cells, validating their biocompatibility. In vivo experiments showed they inhibited microorganism infiltration into wounds and facilitated wound healing. Subcutaneous implantation experiments showed hydrogels degrade over time and restricted inflammatory responses, indicating their compatibility with body tissues (Zhu et al., 2018).

5.1. Bacterial growth inhibition

The antibacterial activity of chitosan-collagen nanoparticle suspension with ZnO was tested using the adapted diffusimetric method by measuring the widths of the zones of inhibition. The best antibacterial activity against S. aureus was achieved for the CS-Coll-ZnO, with the materials formed by precipitation in the presence of NaOH showing a substantial improvement (Tiplea et al., 2021). A mucous adhesive polymeric membrane wound dressing containing hydroethanolic red propolis extract (HERP) was created. Membranes were developed employing a casting method that included collagen, chitosan, polyethylene glycol (15, 20, and 30v %), and a hydroethanolic extraction of EtOH-H_2O 70v% - 30v% (v/v) of HERP (0.5, 1.0, and 1.5%). Membranes demonstrated substantial bacterial inhibition, implying that the 0.5% HERP with chitosan and collagen membrane has the potential for future wound application research. (Loureiro et al., 2020). Silver nanoparticles carrying fibrillar collagen-chitosan hydrogel matrix was developed by biomimetic methodology by mixing silver nanoparticles, collagen fibril and chitosan hydrogel followed by cross-linking and bio mineralization. Antibacterial activity research revealed that *S. aureus* and *E. coli* were inhibited by roughly 27% and 37% of their growth, respectively. In its native state, the developed composite would contain silver nanoparticles loaded with collagen fibril and the resulting bio mineral would be identical to bone mineral. Therefore, the properties and potential of such hydrogel composites could be harnessed for developing biomaterials for applications in bone tissue engineering (Socrates et al., 2019).

5.2. Biocompatible properties

Tissue engineering hydrogels commonly consist of biopolymers due to their distinctive structure and characteristics. The features encompassed are minimal antigenicity and inflammation, strong affinity for water, sufficient cytotoxic effects, compatibility with living tissues, ability to degrade naturally, and a tendency to adhere to mucous membranes (Mozafari et al., 2019). Hydrogels have been found to be effective biomaterials for soft tissue regeneration. Because of its great biocompatibility, Collagen is the primary material used in hydrogel development, but its mechanical strength, temperature resistance, and pH vulnerability make it necessary to explore other options. Collagen and polysaccharides can enhance hydrogel characteristics. Hybrid hydrogels with different collagen/chitosan ratios show comparable physicochemical and microstructural

characteristics, enhancing thermomechanical capabilities and cell viability. This makes them suitable biomaterials for tissue engineering purposes. Hence, the synergism between collagen and chitosan resulted in enhanced hydrogel properties, demonstrating good thermomechanical properties and cell survival for application as promising biomaterials for tissue engineering (Sánchez-Cid et al., 2022). Diabetes mellitus is one of the most common diseases, and it is frequently accompanied by diabetic ulcers. Chitosan, a biopolymer with biodegradability, biocompatibility, and low toxicity, has been used to create hydrogels for diabetic wound treatment. These hydrogels, made of chitosan, collagen, and silver nanoparticles, have been found to have antibacterial, cytotoxic, and swelling properties. In mice with diabetes caused by alloxan, the hydrogels increased the expression of VEGF, TGF-b1, IL-1b, and TIMP1 genes, leading to faster wound healing. The hydrogels also facilitated collagen deposition, hair follicle repair, and sebaceous gland formation. However, clinical trials are needed to fully evaluate the effectiveness of these hydrogels, as animal models do not fully represent the entire diabetic pathology (Shagdarova et al., 2022). Hydrogels made from natural polymers like chitosan and collagen are popular for soft tissue engineering due to their hydrophilicity and softness. However, their mechanical properties require crosslinking techniques like small molecules or synthetic polymers. FTIR-ATR spectra confirmed the expected structure, while scanning electron microscopy showed a porous morphology with interconnected pores. These hydrogels are crucial for soft tissue engineering. Furthermore, swelling degree assay revealed a tunable behavior in natural polymers that was associated with composition. Finally, in vitro biodegradability and biocompatibility experiments demonstrated that the material performed well in simulated biologically living environments with interconnected pores (Duceac et al., 2019). Injectable thermosensitive hydrogels made of chitosan, collagen, and β-GP were used, which can form a gel at body temperature and are suitable for treating uneven surface wounds. These hydrogels offer a cell-friendly environment like the extracellular matrix (ECM) in cell-based therapies and are compatible with living tissues. Collagen in the hydrogel facilitates cell adhesion, migration, survival, and proliferation, and encourages gel remodeling. The hydrogel, combined with 3D MSC spheroids, significantly influenced the growth and release of paracrine factors, enhancing its effectiveness in healing deep skin wounds by stimulating the growth of new blood vessels and skin regeneration (Yang et al., 2020).

5.3. Wound healing in moist environments

Hydrogels, which are classified as advanced dressings, possess the ability to sustain a moist environment at the specific location of application. This characteristic, attributed to their high-water content, renders hydrogels very suitable for the purpose of wound treatment. Hydrogels have the potential to be utilized in the treatment of both exudating and dry necrotic wounds (Gupta et al., 2019). Moist conditions are crucial in the management of wound healing acceleration. In contrast to scaffolds of diverse compositions, hydrogels possess the ability to sustain a wet environment within the wound region. Cross-linked hydrophilic polymeric networks that bear resemblance to natural soft tissues and extracellular matrix are present (Li et al., 2020). The process of wound healing is a multifaceted biological phenomenon that encompasses the regeneration of damaged tissue. Conventional wound dressings exhibit a lack of moisture retention, hence impeding the creation of an optimal moist environment conducive to wound healing. Additionally, these dressings demonstrate limited efficacy in terms of their antibacterial capabilities. Hydrogels possess the ability to retain substantial quantities of water, hence facilitating the establishment of a moist healing environment. At present, phototherapies have demonstrated significant potential in the realm of bacterial illness treatment. Hence, the integration of hydrogels with phototherapy presents a viable solution to address the limitations associated with conventional approaches to wound management. This combination exhibits considerable promise in promoting wound healing due to its notable efficacy, little irritability, and favorable antibacterial properties (Y. Xu et al., 2022).

5.4. Hemostasis

Development of a composite sponge made of halloysite, chitosan, and collagen using directed freeze-drying techniques was studied. The sponge was then coated with a hydrophobic polydimethylsiloxane layer to enhance its hemostatic properties. The sponge's channel structure, with a pore size of 30 μm, facilitates blood transportation. The sponge's morphology and spectrum analysis show that chitosan and collagen adsorb onto the external surface of HNTs due to hydrogen bonding and electrostatic attraction. The directional freeze-dried sponge showed faster blood absorption rates than its non-directional counterpart. The composite sponges also showed significant antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus*, were non-cytotoxic to mouse fibroblasts, and showed excellent compatibility with blood cells. The use of the hemostatic dressing effectively mitigated superfluous blood loss due to its exceptional capacity for excessive blood absorption. The efficacy of the asymmetric sponges in promoting quick clotting and minimizing blood loss was validated in in vivo trials on rats. The study highlights the potential of this dressing in wound healing (Lin et al., 2023). Numerous chemicals have been identified as potential

hemostatic agents, although their efficacy is contingent upon the specific nature of the bleeding event and the anatomical site of the laceration or injury. Although there are numerous effective hemostatic materials available for treating superficial wounds that can be compressed, managing internal vascular and surgical bleeding continues to pose challenges. The difficulties about strong adherence in wet environments and applications in complex conditions, such as cutaneous, cardiac, and liver lesions, have been effectively addressed by recent advancements in wet-adhesive hydrogels. These hydrogels can also be biocompatible, antimicrobial, and high blood pressure resistant, outperforming certain conventional products (Han & Wang, 2023). The utilization of hydrogel bio adhesion technology has presented remarkable prospects in the field of minimally-invasive operations, which are commonly conducted with the aim of mitigating postoperative complications, shortening recovery periods, and alleviating patient suffering. Current hydrogel-based adhesives have challenges due to their limited ability to adhere in wet and dynamic environments, as well as potential immunological adverse effects, particularly in the case of synthetic hydrogel bio adhesives. A novel class of synthetic hydrogel bio adhesives derived from diverse polymer precursors hase been presented. These bio adhesives exhibit rapid creation of a durable bio interface, enabling effective adherence to moist and resilient biological tissues with significant mobility. Furthermore, the elimination of monomers in the process of hydrogel manufacturing ensures that these hydrogel adhesives do not elicit an inflammatory response when used for in vivo wound sealing. This property has great potential for the prompt healing of vascular defects and surgical hemostasis. Furthermore, these materials could potentially function as interfaces between humans and electronic devices, facilitating the integration of bioelectronics implants for the purpose of monitoring physiological and clinical data in real-time (Zhang et al., 2022).

VI. ANTI-MICROBIAL AND WOUND HEALING APPLICATIONS OF CHITOSAN-COLLAGEN BASED HYDROGELS

6.1. Controlled release of antimicrobials and drugs

Researchers aimed to create composite hydrogels by combining blue crab chitosan (CS) with bluefin tuna collagenous protein (BTCP) at different concentrations. The hydrogels showed improved porosity and swelling degree with increased BTCP concentration. The hydrogels also showed enhanced elasticity and mechanical strength, particularly in hydrogels with 50% BTCP. The pH of the surrounding medium impacted the release of phycocyanin, a biologically active chemical. The study suggests that pH-sensitive Cs-BTPC composite hydrogels may provide a favorable environment for encapsulation and delivery of medicinal drugs. The study presents a new method for promoting maritime industries by focusing on the utilization and economic significance of marine by-products, known as the blue economy. The chitosan-collagen combination is recognized as a highly effective substrate for developing materials with significant potential in tissue engineering and drug delivery applications. The physicochemical properties of the hydrogels were assessed using Fourier Transform Infrared Spectroscopy, X-ray Diffraction, and Scanning Electron Microscopy (Azaza et al., 2023).

As discussed previously, hydrogels can be formulated to deliver drugs using passive and active mechanisms, and hydrogel coatings can use these strategies to provide local and sustained release of antibacterial compounds to prevent implant infections. For example, the porosity of hydrogel coatings can be tuned to create “active” coatings that release small molecules or particles embedded in the scaffold. One strategy being explored clinically is the encapsulation of intrinsically antimicrobial silver nanoparticles into polyacrylamide-based hydrogels to prevent infections from E. coli and S. aureus (Qasim et al., 2018).

Thermosensitive hydrogels called bTCP-chitosan/collagen-quercetin hydrogels were prepared by combining beta-tricalcium phosphate (bTCP) nanoparticles and quercetin with a chitosan/collagen composite. These hydrogels undergo a sol-gel transformation triggered by beta-glycerophosphate (bGP) and changes in temperature, aligning with typical body temperature and pH conditions **(Fig. 11)**. The hydrogels showed a porous structure with interconnected pore architecture. The addition of 3% bTCP improved the hydrogels' mechanical characteristics, decreased swelling and degradation rates, and improved pore size, permeability, and quercetin release rate. The hydrogels were biocompatible and showed the ability to support cell encapsulation. The consistent and ongoing release pattern of quercetin from the 3% bTCP-hydrogel suggests it could be an effective carrier for delivering natural flavonoids for bone repair (Sareethammanuwat et al., 2021).

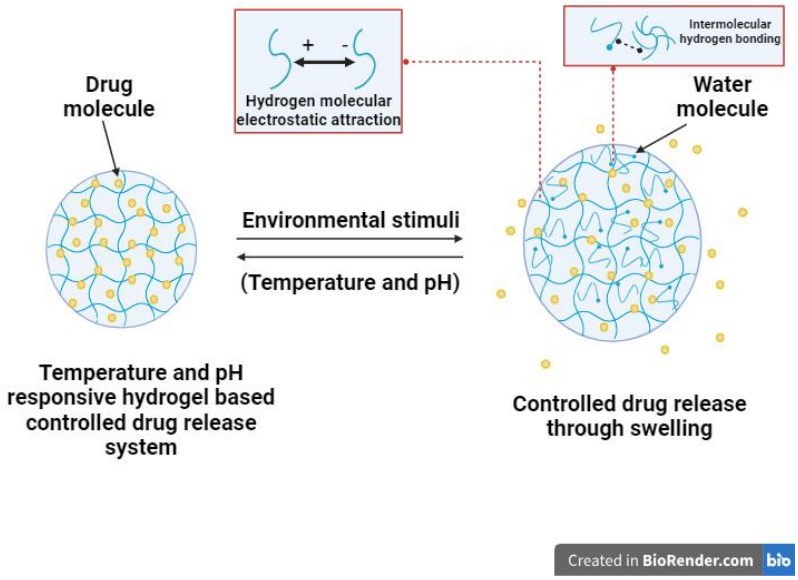

Fig.11: Controlled drug release

Biopolymer hydrogels were created by combining hyaluronic acid, hydrolyzed collagen, and chitosan with caffeic acid as an antioxidant agent. The hydrogels were characterized using X-ray diffraction, differential scanning calorimetry, and thermogravimetric analysis. The hydrogels showed no structural or thermal changes, and their swelling behavior was superior due to high hyaluronic acid concentration. The initial rapid release of caffeic acid was around 70% within 60 minutes, followed by a slow release of up to 80% by 480 minutes. The antioxidant activity of the hydrogels was demonstrated through DPPH, ABTS+, and FRAP tests, suggesting their potential as dressings (Chuysinuan et al., 2020).

Hydrogel composites made of collagen and chitosan were synthesized using a solvent casting method, with caffeic acid (CA) as a crosslinking agent. The hydrogel's structural characteristics were studied using FTIR spectroscopy. The presence of CA impeded the molecular chain mobility of chitosan and collagen, causing cracking and dimensional stability issues. The addition of CA reduced swelling properties and degrading behavior. The drug release profile showed a progressive pattern over 8 hours, influenced by CA quantity. The composite hydrogel's antioxidant activity was evaluated, indicating its potential in drug release and cosmetic research (Thongchai et al., 2020).

A porous bio-sponge for oral mucositis treatment was developed by blending collagen with chitosan. The impact of blending on crystallinity structure and thermal behavior of both polymers was evaluated. In vitro studies showed a semi-crystalline structure in the collagen-chitosan film, which significantly impacts drug release. FT-IR analysis revealed electrostatic interactions and hydrogen bonding between collagen and chitosan, as well as between dexamethasone and the polymers. The collagen-chitosan (1:1) blend formulation effectively regulated medication release within a 10-hour timeframe, compared to the collagen sponge's completion time of 16 hours (Alagha et al., 2020).

6.2. Surgical site dressings

Hydrogel coatings provide novel strategies to combat one of the biggest challenges for implantable devices infections. In 2011 alone, the United States saw nearly 185,000 cases of hospital-acquired infections associated with medical devices (Magill et al., 2014). The use of mesh in hernia repair procedures relates to a significant risk known as surgical-site infection **(Fig. 12)**. Triclosan-infused absorbable suture materials are used to reduce the occurrence of infections. To enhance the therapeutic efficacy, an experiment was conducted whereby the efficacy of using meshes coated with chitosan gel containing triclosan was examined to prevent and treat mesh infections in a rat model. A model of mesh infection was established using simultaneous and 24-hour Staphylococcus aureus injection. The rats were then monitored for a duration of 8 days to assess the occurrence of surgical-site infections. According to the findings, grafts that were covered with chitosan gel containing triclosan shown effective preventative benefits against graft infection (Çakmak et al., 2009).

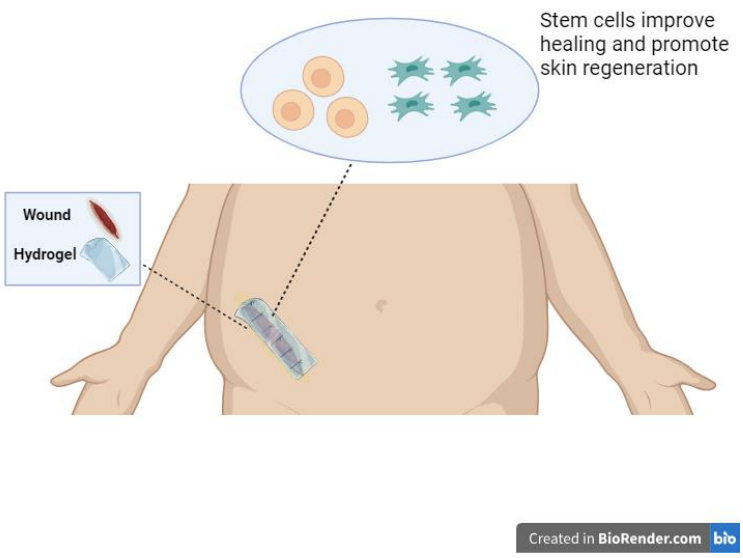

Fig.12: Use of hydrogels as surgical dressing for appendicitis surgery wound

The adhesion of laser-activated chitosan films in a mixed in-vitro and animal setting was investigated. The purpose of this investigation was to explore the potential applicability of these films for suture-less tissue fixation. Sheep intestine was affixed with chitosan films, which were in the form of flexible and insoluble strips. This bonding process was carried out utilizing laser energies of varying intensities, specifically at a wavelength of 808 nm. In addition, genipin, a natural cross-linker, was incorporated into the film to assess its impact on tissue restoration strength in comparison to the films that were plain. The dressing was also applied in vivo to the sciatic nerves of rat models, and the resulting thermal damage caused by the laser was assessed four days post-surgery. The experimental findings provided evidence that the application of chitosan adhesives effectively facilitated the restoration of intestinal tissue, with the highest level of strength of repair observed at a 120-mW laser power. The chitosan gel loaded with n- showed effective preventative benefits against graft infection (Lauto et al., 2007).

A composite dressing made of collagen, chitosan, and alginate was developed to improve wound healing and resist seawater immersion. The CCA cushion was fabricated using paint coat and freeze-drying methods and attached to a polyurethane material. The dressing showed favorable water absorption and mechanical properties, resulting in a higher wound healing ratio in rats compared to gauze or chitosan treatment. The dressing also showed increased fibroblast cells, intact re-epithelialization, and increased levels of EGF, bFGF, TGF-β, and CD31. The dressing showed no harmful effects on cells and showed positive compatibility with blood (Xie et al., 2018).

The chitosan/βGP hydrogel is made and subsequently combined with a liposomal formulation of diethyldithiocarbamate and copper ions for the purpose of treating surgical site infections. The injectable gel has a high efficacy in eradicating 98.7% of methicillin-resistant Staphylococcus aureus and effectively inhibiting 99.9% of biofilm formation caused by Staphylococcus epidermidis within a 48-hour timeframe (Kaul et al., 2022).

A surgical wound refers to a deliberate cut created by a qualified physician. The delayed process of surgical wound healing has the potential to result in the development of chronic wounds, which can pose a significant health concern. This research aims to improve the healing process of surgical wounds by developing liposomes containing curcumin within a hydrogel made of lysine and collagen. The liposomal formulation was prepared using the thin-film hydration method and characterized for size, shape, encapsulation efficiency, and in vitro release. The hydrogel matrix was created, followed by the infusion of curcumin-loaded liposomes, resulting in formulations F1, F2, and F3. Safety, stability, swelling index, pH, rheological characteristics, and in vivo wound healing assay were used to characterize all formulations. The histology and histomorphometry of tissue samples from the wound area were also analyzed to assess the effects of the formulations, as well as the control group. This suggests how collagen hydrogel address the potential health concerns associated with delayed surgical wound healing (Cardoso-Daodu et al., 2022).

Surgical infections can lead to delayed wound healing, oxidative stress, and tissue ischemia. Hydrogels, non-antibiotic wound dressings, have potential to address these issues. A reductionism methodology has been

developed to produce bioactive hydrogels with antibacterial, antioxidant, pro-angiogenic, and hemostatic properties. These hydrogels, made from extracts from Cirsium setosum (CE) and carboxymethyl chitosan (CS), have been tested in three models. The hydrogels showed significant efficacy in mitigating bleeding and enhancing vascularization for skin flap regeneration, demonstrating the potential of these hydrogels. In general, the integration of bioactive CECS hydrogels with a straightforward and scalable assembly technique, as well as their inherent biological activities without the need for antibiotics, holds promise for their application as multifunctional wound dressings in surgical anti-infection procedures (Geng et al., 2022).

6.3. Burn care.

Burn injuries are a common and severe type of trauma that necessitates comprehensive medical attention for patients. The prompt administration of medical intervention for burn injuries has been shown to have a substantial positive impact on the process of wound healing. The effectiveness of amnion and collagen-based hydrogels in promoting the healing of cutaneous burn wounds in rats was studied. A unique cell-free hydrogel was formulated using a combination of human amnion, rabbit collagen, carboxymethyl cellulose sodium salt, citric acid, methyl paraben, propyl paraben, glycerin, and triethanolamine. The wound dressing material was created using a combination of rabbit collagen and chitosan derived from prawn shells. The hydrogels were found to be non-cytotoxic and compatible with human blood cells. The gels accelerated wound healing, with complete re-epithelialization occurring within 16.75 ± 0.96 days and wound closure via contraction reaching 72 ± 3.27% when a wound dressing membrane was applied. The sprayable hydrogel with a covering membrane was more effective **(Fig. 13)**. This suggests a potential alternative for treating cutaneous injuries, addressing issues like high costs and logistical intricacies (Rana et al., 2020).

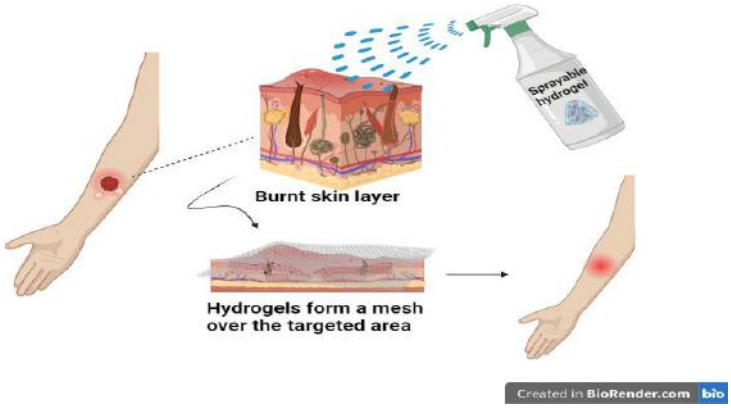

Fig.13: Burn wound healing

A study was conducted on the effectiveness of the HemCon™ bandage in treating burn infections in mice. The study found that the bacterial population in untreated burns increased by about 1000-fold from day 0 to day 3. However, in burns treated with silver dressing or chitosan acetate bandage, the bacterial luminescence signal decreased on day 1. The chitosan acetate bandage had a higher survival rate for Pseudomonas aeruginosa infections, with a survival rate of 73.3%. The chitosan acetate bandage was found to be effective in managing bacterial growth in burn wounds and preventing systemic sepsis, as confirmed by quantitative analysis of bacterial luminescence signals and blood culture results (Dai et al., 2009).

Utilizing a rat model to examine and compare the impact of chitosan and heparin on the first expansion of burn injuries, a study was conducted (Jin et al., 2007). A study on rats showed that chitosan powder, heparin powder, and a combination of chitosan and heparin were used to induce burns. The results showed that chitosan had a lower severity of burns and a significant preventive effect on the initial phase, while heparin did not. However, concurrent administration of chitosan and heparin reduced chitosan's protective efficacy. The efficiency of chitosan in promoting wound healing in rat burn injuries was assessed (Burkatovskaya et al., 2006).

Lysostaphin was investigated against MRSA-induced burn infection in New Zealand White rabbits by combining it with a chitosan-collagen hydrogel (CCHL). The scientists created third-degree burn wounds (3 × 3 cm) using an electronic temperature controller set to 80 °C for 15 seconds. Two days after the burning, the eschar was removed, and each wound contained MRSA (200 L of 1 109 CFU/mL bacterial solution). When compared to groups treated with chitosan-collagen hydrogel without lysostaphin or animals treated with saline, the animals treated with CCHL should exhibit improvements in lesion healing

coupled with a decrease in MRSA burden. Additionally, after therapy, the administration of CCHL resulted in better repair of tissue architecture (Cui et al., 2011).

A chitosan hydrogel was developed for use as a wound dressing for burn wounds in rats. The hydrogel facilitated cell adhesion and proliferation and showed no harmful effects. The wound beds of the treated animals were significantly smaller than the untreated control group. Histological examination showed no inflammatory responses in the skin lesions treated with the hydrogel, and no pathological abnormalities were observed in the organs retrieved during necropsy. These findings suggest the biomaterial's compatibility at both local and systemic levels (Ribeiro et al., 2020).

The effect of a thermosensitive hydrogel made from chitosan, collagen, and β-glycerophosphate (β-GP) and a conditioned medium from human umbilical cord mesenchymal stem cells (MSC-CM) on mice with third-degree burns was examined. The hydrogel was stored at 4°C and applied to the wounds of the mice. The mice were divided into three groups and treated with different treatments: unconditioned MSC medium, MSC-conditioned medium, or a combination of unconditioned medium with chitosan, collagen, and β-GP thermosensitive hydrogels. Skin tissue samples were collected at different time points and analyzed using hematoxylin and eosin staining and Ki-67 staining. A comparative analysis was conducted to assess the rates and durations of wound healing within the four experimental groups. The use of MSC-CM/hydrogel showed several beneficial effects, including reduced healing time, inflammation restriction, epithelialization facilitation, well-vascularized granulation tissue development, and mitigating the creation of fibrotic and hypertrophic scar tissue. In summary, the use of MSC-CM/hydrogel shows significant efficacy in facilitating wound healing in mice with third-degree burns (Zhou et al., 2019).

Marine organisms yield natural compounds that contain biologically active substances, such as collagen, which serve as a significant reservoir of compounds with medical potential. These compounds have been found to be particularly useful in wound dressing applications, especially when used in conjunction with other natural or synthetic materials, since they expedite the wound healing process. This study evaluates the capacity of collagen derived from Rutilus kutum skin combined with chitosan in a collagen-chitosan gel for facilitating the recovery of second-degree burn injuries in rats. Collagen and chitosan were extracted from discarded skin and shells of prawns, and their subunit composition was evaluated using SDS-PAGE. High-performance liquid chromatography (HPLC) was used to analyze amino acids, and different Col-CH gel formulations were prepared in different ratios. The results showed a significant reduction in wound size in Col-CH treated animals compared to silver sulfadiazine ointment. Histological examination showed an enhancement in epithelial cell growth and blood vessel growth, and a decrease in inflammatory cells. The data suggests that the Col-CH gel combination effectively cures burn wounds, surpassing silver sulfadiazine ointment (Naderi Gharehgheshlagh et al., 2021).

The effects of collagen-chitosan gel from Scomberomorus guttatus and prawn skin on second-degree burn healing in rats was investigated. Results showed that Col-CH (3:1) treatment significantly decreased wound size and increased epithelialization, collagen content, and fibroblast cell presence. It also reduced inflammatory cell infiltration. The study concluded that Col-CH gel showed superior effectiveness in promoting burn wound healing on the 25th day after the burn, compared to sulfadiazine (Fatemi et al., 2021).

Chronic wounds can lead to limb amputation, and researchers have explored pro-angiogenic agents like cerium oxide and cerium peroxide nanoparticles. These nanoparticles were incorporated into chitosan and collagen hydrogel matrices, and their pro-angiogenic characteristics were investigated using in-vivo CAM tests. The study found that the presence of cerium peroxide in the hydrogels significantly enhanced angiogenesis, compared to cerium oxide-loaded materials. This suggests that cerium peroxide incorporated chitosan and collagen hydrogels have potential for facilitating the healing process of chronic ulcers and burn wounds (Zubairi et al., 2022).

The demand for wound dressings for partial-thickness burns is increasing, and hydrogels have potential as materials for sustaining hydration and facilitating tissue elimination. A study incorporated tilapia peptides and hydroxyapatite into a chitosan system to create novel hydrogels. These hydrogels showed exceptional water absorption, minimal hemolysis, and antibacterial efficacy against bacteria. They also promoted skin regeneration by decreasing TNF-α and IL-6 expression (Qianqian et al., 2021).

6.4. Wound dressings and wound healing

The impact of collagen on chitosan/gelatin hydrogels, specifically their growth and attachment of fibroblasts for wound dressing applications was studied. The hydrogels were synthesized using chitosan and collagen biopolymers, with varying ratios. The hydrogels showed improved mechanical properties, reduced swelling ratio, and superior water vapor transmission rate. Collagen also resulted in a uniform and interconnected architecture,

leading to higher cell survival and attachment rates. These findings suggest the potential of chitosan/collagen hydrogels for wound dressing applications (Mousavi et al., 2019).

One of the most appealing options for a wound dressing is hydrogel because, in addition to acting as a barrier against microorganisms, it also maintains a moist environment at the wound interface, offering three-dimensional structures that support cell adhesion and proliferation and permit the exchange of gases, nutrients, and metabolic waste products (Lakshmanan et al., 2013). A study involving water-soluble carboxymethyl-chitosan (CMCS) crosslinked with genipin was conducted to investigate its impact on wound healing properties. The CMCS was divided into hydrogel, membrane, and sponge dressings. The sponge dressing showed superior water absorption, gas permeability, hemostatic performance, and promotes skin fibroblast proliferation. It also induced matrix metalloproteinase-1 production. The sponge dressing was effective in wound closure and expedited healing in in-vivo settings. The hydrogel and membrane showed biocompatibility, hemostatic characteristics, and wound healing facilitation. (D. Wang et al., 2020).

A novel chitosan-collagen sponge (CCS) was developed with the intention of exploring its application as a biomaterial for wound dressings. Wound dressing was prepared by using a combination consisting of 3.0% chitosan and 1.0% type I collagen in a ratio of 7:3 (w/w). This mixture was subjected to the freeze-drying process. Subsequently, the dressing was made to assess its qualities via a sequence of tests. The newly developed dressing exhibited its ability to ensure the safety of NIH3T3 cells. The study found that chitosan-collagen wound dressing showed significant improvement in healing after a surgical procedure **(Fig. 14)**, indicating its potential as a viable option for future wound treatments. (Zhang et al., 2021).

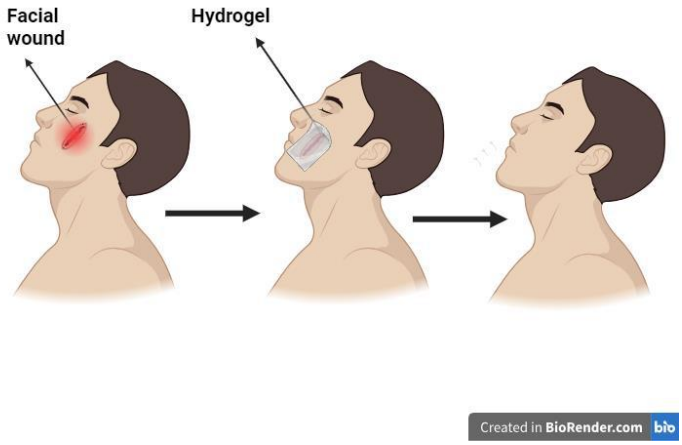

Fig.14: Hydrogels are used in faster healing of facial wounds

The equine distal limb wound healing model, similar to human wound healing, is used for investigating biomaterials with potential applications in veterinary and human medical fields. A study found significant differences in functional and structural aspects of unwounded and injured skin across different locations on the distal limb. The study also examined the impact of a collagen-chitosan hydrogel modified with peptides on wound healing. The Q-peptide hydrogel increased wound closure and modulated the biomechanical properties of the healed tissue, resulting in a more compliant structure (Sparks et al., 2021).

The use of alginate dialdehyde (ADA) as a crosslinker was explored to improve the mechanical properties of collagen-chitosan (COL-CS) membranes. The resulting COL-CS-ADA films showed improved thermal stability and mechanical characteristics, increased cross-linking with higher oxidation levels, and no harmful effects on fibroblasts. Additionally, the ADA film significantly improved wound healing and biocompatibility (Yang et al., 2019).

Wound dressings, including hydrogels, films, wafers, nanofibers, foams, and transdermal patches, are used to treat both acute and chronic wounds. The utilization of chitosan and cellulose, two biopolymers, in the formulation of hydrogels was examined with the aim of enhancing wound management. Hydrogels have unique properties like a damp environment, moisture retention, and bacterial protection. Biopolymers, like cellulose and chitosan, are used due to their non-toxic, biodegradable, and biocompatible properties. These hydrogels accelerate wound healing, mimic skin structure, and facilitate skin

regeneration. Antibacterial compounds also help prevent microorganism entry. (Alven & Aderibigbe, 2020).

A collagen and chitosan composite gel was developed, incorporating oligoarginine (R8) as a cell penetrating peptide. The gel was examined for its physical and chemical properties using various techniques. Results showed that the gel effectively inhibited Staphylococcus aureus proliferation and promoted wound healing. The gel showed the highest healing rate and rapid speed compared to other treatments. It also enhanced skin wound healing by facilitating granulation tissue formation, collagen accumulation, and angiogenesis (M. Li et al., 2019).

The properties and compatibility of a hydrogel made from collagen, chitosan, and dialdehyde starch, Col/Ch/DAS were investigated. The hydrogels were created by combining collagen and chitosan, with a cross-linker called DAS. The hydrogels showed high swelling and biodegradability and were suitable for wound dressings. A collagen and chitosan composite gel was also created, which inhibited Staphylococcus aureus proliferation and promoted wound healing. The gel showed remarkable healing rates and speed, enhancing skin wound healing by facilitating granulation tissue formation and collagen accumulation (Valipour et al., 2023).

Wound healing dressings and scaffolds were introduced, which were composed of single layers in the form of electrospun sheets and hydrogels. A bilayer scaffold consisting of chitosan/gelatin hydrogel and co-electrospun PCL/PVA was developed. The uniqueness of the product stemmed from the utilization of the freeze-gelation technique to attain a porous structure within the hydrogel component of the bilayer configuration. The qualities of the product and its performance in wound healing were evaluated by various methods, including scanning electron microscopy, MTT proliferation assay, swelling analysis, tensile strength testing, and in vivo assessments (Kamali & Shamloo, 2020).

6.5. Regeneration of tissues

The utilization of hydrogels derived from various materials has emerged as a novel strategy within the biomedical domain, namely in the realm of regenerative medicine (Catoira et al., 2019). Nerve regeneration using a chitosan-collagen hydrogel neural conduit (CCN) encapsulating Schwann cells (SC) in a rat model with sciatic nerve defects was investigated. The CCN+ group showed enhanced motor functional recovery, axonal regeneration, and myelination compared to the CCN- and silicone+ groups. The use of SC-encapsulated CCNs demonstrates a collaborative impact on peripheral nerve regeneration, promoting the development of axons and remyelination of host SCs. This suggests SC-encapsulated CCNs could be a viable strategy for addressing extensive peripheral nerve deficits (Takeya et al., 2023).

The production of porous chitosan/collagen composite scaffolds for peripheral nerve tissue engineering applications was studied. The scaffolds were examined using various analytical techniques, including shape, porosity, liquid absorption capacity, swelling behavior, composition, mechanical properties, and degrading behavior. The composite scaffolds showed surface morphology resembling fibers and internal porosity, with chitosan reducing pore size, liquid absorption capacity, and rate of degradation. The physicochemical features were suitable for the proposed use, and the composite scaffolds exhibited cytocompatibility without any harmful effects. The study also demonstrated the composite scaffolds' ability to enhance Schwann cell adhesion, movement, and replication. The chitosan/collagen composite scaffolds have considerable potential for peripheral nerve regeneration **(Fig. 15)** (Si et al., 2019).

The investigation focused on the potential application of hydrogels composed of chitosan and collagen in the field of skin tissue engineering. Three amino acids (arginine, alanine, and phenylalanine) were incorporated into chitosan/collagen hydrogels, ACC hydrogels. The ACC hydrogels were synthesized using freeze drying and assessed for their angiogenic capabilities using the chorioallantoic membrane assay. ACC hydrogels loaded with arginine had the highest porosity and the highest formation of blood vessels. CH-Arg hydrogels showed higher efficacy in promoting angiogenesis compared to control materials (Aleem et al., 2019).

Fucoidans, sulfated polysaccharides from brown algae, are essential for bone regeneration. To use them in regenerative medicine, a delivery system that temporarily immobilizes the polysaccharide at the injury site is crucial. Hydrogels, which are less invasive, have gained attention for their ability to facilitate healing processes. This study aimed to develop an injectable thermosensitive hydrogel for fucoidan delivery using chitosan, collagen, and β-glycerophosphate. The hydrogel showed compatibility with mesenchymal stem cells (MSC) and endothelial cells (OEC), demonstrating their potential in regenerative medicine. Collagen and chitosan are also recognized biomaterials with their inherent characteristics, functional properties, and environmental friendliness. A study on Col/Ch/DAS hydrogels found that the composition of DAS significantly influenced swelling ratio and biodegradability. The gel composite showed antibacterial characteristics, indicating its therapeutic effectiveness in wound healing (Ohmes et al., 2022).

A hydrogel composite scaffold was created using chitosan-collagen hydrogel, 3D printed poly(lactic acid) struts and nanofibrous cellulose. The scaffolds, which were shaped into micro- and nano-sized topographical features, were designed to enhance cellular activities and mechanical characteristics. The scaffolds, which were applied with different concentrations of genipin, showed an interconnected microporous architecture, a swelling ratio of 400%, and a compressive strength of approximately 32 kPa. The scaffold showed minimal cytotoxicity towards rabbit mesenchymal stem cells and facilitated cellular attachment, proliferation, and migration (Gunes et al., 2022).

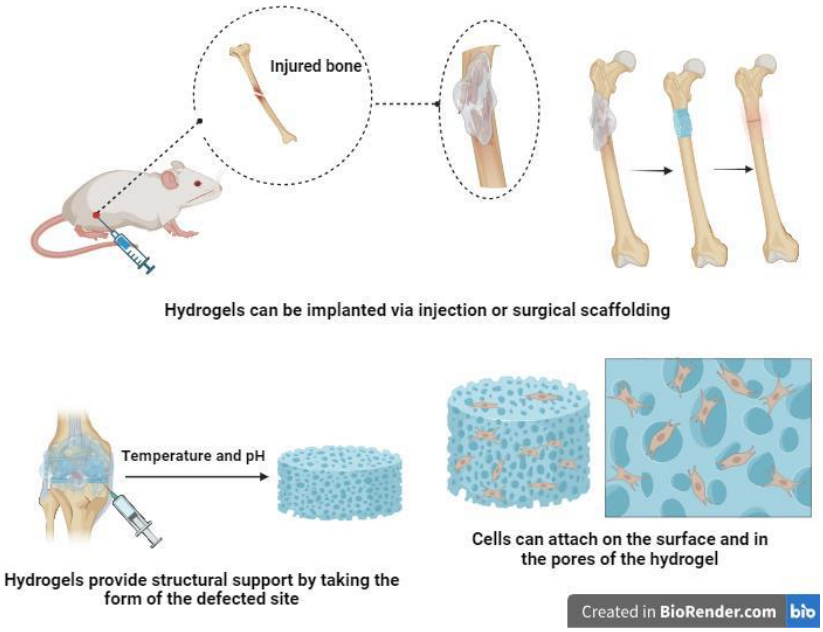

Fig.15: Hydrogels help in bone and tissue repair

The use of crosslinking strategies in creating polymeric biomaterial scaffolds was examined. It focuses on a collagen-chitosan hydrogel film created using tannic acid and genipin simultaneously. The film's porosity and strength were evaluated using infrared analysis spectroscopy, scanning electron microscopy, and thermo-gravimetric analysis. The dual crosslinking process significantly influences the films' strength and adhesion and multiplication of cells. This technique has gained widespread use in ophthalmology for temporary corneal injuries and skin tissue regeneration (Shah et al., 2019).

The hydrogel nanocomposites were fabricated using chitosan/collagen and chitosan/collagen/nano-hydroxyapatite (nHAP) from Persian Gulf shrimp wastes and rat tail tendons. The porous scaffolds were assessed using various techniques like SEM, FTIR, water content, STA, and AFM nanoindentation. The results showed significant promise for cartilage tissue engineering, suggesting the hydrogels could be a viable candidate (Kaviani et al., 2019).

The skin is a multilayered organ that acts as the primary barrier between the inside tissues or cells and the outside environment, protecting them. To achieve the desired mechanical qualities of artificial skin, it is imperative to priorities the considerations of biocompatibility and biodegradability. In contrast to other living organisms, humans have exhibited limited tissue regeneration due to genetic variability. Furthermore, hydrogel scaffolds have gained a growing interest for the purpose of repairing and regenerating skin tissue. This is primarily due to their remarkable capacity to undergo self-renewal and promote the proliferation of the specific cell populations that play a crucial role in the regeneration of skin tissue. Additionally, an investigation was conducted on the intelligent healing capabilities of hydrogel scaffolds loaded with peptides and growth factors for the purpose of regeneration in skin tissues (Kalai Selvan et al., 2020).

ColChHAmod (Collagen-Chitosan-Hyaluronic acid) hydrogels, made from genipin crosslinked collagen, chitosan, and lysine-modified hyaluronic acid, are a novel material for injection applications. These hydrogels have unique properties, such as interaction with living organisms and multiple functions. By adding primary amine groups through lysine attachment, HAmod can form covalent links with other hydrogel components, resulting in structurally robust and clear hydrogels. The hydrogels can be controlled for bone tissue regeneration and have varying physicochemical properties. In vitro cell culture experiments confirmed their biocompatible surfaces and

antibacterial activity against Escherichia coli (Gilarska et al., 2020).

Use of alkaline phosphatase (ALP) in nanotubes made of halloysite (HAL) and incorporated into hydrogel scaffolds made of chitosan (CH) and chitosan-collagen (C-CH) to promote bone regeneration was studied. The addition of 30% HAL-ALP significantly increased the swelling ratio of chitosan-based scaffolds, while collagen enhanced porosity. The biomineralization process was more effective in hydrogels with collagen. C-CH scaffolds, particularly those with biomineralization, showed enhanced cell attachment and proliferation properties. The C-CH scaffolds with a 30% concentration of HAL-ALP showed the highest capacity for bone regeneration (Pietraszek et al., 2020).

Biomineralization is crucial for bone repair, where calcium and phosphate ions are deposited into the extracellular matrix (ECM). To mimic this process, a composite hydrogel made of chitosan and collagen has been created. The hydrogel contains black phosphorus coated with mesenchymal stem cell membranes, which activates osteoblast recruitment through near-infrared light. This leads to the formation of hydroxyapatite, which stimulates osteoblast movement and bone formation. The hydrogel therapy, when implanted into cranial defects of lab rats, improved local bone density and new bone generation. This research has significant implications for bone healing and treating cranial abnormalities in clinical settings (Tan et al., 2022).

6.6. Management and healing of ulcers

A porous cross-linked hydrogel was developed for treating persistent skin ulcers, combining collagen and chitosan. Cross-linking techniques like UV irradiation, tannic acid, and ultrasonication were used. The hydrogel's composition, chitosan concentration, and ratio were crucial for its effectiveness. Stable systems were achieved through freeze-drying. The Design of Experiments methodology was used to determine the optimal hydrogel composition. The scaffold's biocompatibility, biomimicry, and safety were tested in vitro and in vivo (Valentino et al., 2023).

Diabetic foot ulcers (DFUs) represent a highly widespread concern commonly observed in individuals diagnosed with diabetes mellitus. Diabetic foot ulcers (DFUs) are enduring lesions that frequently result in non-traumatic amputations of the lower extremities, primarily due to persistent infection and other adverse effects associated with ulcers. Furthermore, these issues pose a substantial financial strain on the healthcare system due to the necessity of costly medical interventions. Furthermore, the existing clinical interventions for diabetic foot ulcers (DFUs) have demonstrated only modest efficacy, highlighting the urgent requirement for the development of innovative approaches to enhance the treatment outcomes of DFUs. Hydrogels are complex structures that can be produced using a combination of natural and/or synthetic polymers. These materials have undergone substantial research for many biological applications, such as drug delivery and tissue engineering, due to their distinctive adaptability, tunability, and hydrophilic characteristics (Güiza-Argüello et al., 2022).

Based on data provided by the World Health Organization, an estimated annual fatality counts of 180,000 has been attributed to burn-related incidents. Chronic wounds affect 6.5 million Americans, requiring advanced therapeutic strategies. Biodegradable polymeric wound dressings have been developed over the past 50 years, eliminating the need for frequent replacements, and reducing immune response risk. These polymers come from natural or synthetic sources, with natural polymers preferable due to biocompatibility and mechanical capabilities (Miguel et al., 2021).

Diabetic foot ulcers (DFU) frequently exhibit a challenging healing process, even when conventional care protocols are implemented. The effectiveness of novel collagen matrix dressings was assessed using chitosan-collagen hydrogel in comparison to conventional dressing, for the purpose of promoting wound healing in individuals with a chronic diabetic foot ulcer (DFU). The study involved 61 patients with neuropathic diabetic foot ulcers. The study group received a collagen matrix dressing from Tebaderm, while the control group received gauze. The study group showed a higher reduction in DFU size at four weeks and a higher percentage of full healing at 20 weeks. Collagen matrix dressings can expedite wound healing, potentially reducing the duration needed for full recovery (Djavid et al., 2020).

Corneal disease is the second leading cause of blindness, with around 10 million people worldwide experiencing visual impairment. Research on wound dressings for corneal coverage has shown potential for optimal healing. A chitosan coating was applied to an electrospun membrane made of collagen, hyaluronic acid, and PEO, with glutaraldehyde as a crosslinking agent. The coating enhanced membrane transparency but did not significantly change it. The pore diameter of the membrane decreased with increased chitosan coating concentration. The membrane's cell viability was non-toxic, and the coating showed antibacterial properties, particularly against Pseudomonas aeruginosa. In summary, the incorporation of a chitosan coating has been found to enhance the properties of the electrospun membrane utilized for wound dressing in cases of corneal ulcers (Putra et al., 2020).

The most effective delivery strategy for mesenchymal stem cells secretome as a diabetic wound dressing is a film-forming spray composed of a hydrogel matrix including carboxymethyl chitosan, hyaluronic acid, and collagen tripeptide (CCHACTP). The application of a chitosan/collagen hydrogel patch resulted in the expedited healing of diabetic foot ulcers (Umar et al., 2022). Chitosan-based hydrogels and membranes are used for managing cutaneous injuries like wounds, burns, and ulcers. They improve wound healing by boosting immunomodulatory, antimicrobial, and local cell proliferative activity. They stimulate cellular metabolism and reduce scar size. Chitosan-based membranes have favorable characteristics, making them suitable for chronic wound management. Changes to chitosan can improve its physicochemical properties, creating multilayer membranes and self-healing hydrogels. Other formulations include sponges, topical gels, and coatings (Kim et al., 2023). Studies have been conducted on chitosan biomaterials for the purpose of treating wounds with diverse causes, such as burn wounds and pressure ulcers. These biomaterials are utilized in the development of dressing materials. (Zavyalova et al., 2021).

6.7. Closure of surgical wounds

Traditionally, conventional methods for closing surgical wounds have involved the utilization of sutures, staples, or wires. Nevertheless, the use of these methodologies, specifically in relation to parenchymatous tissues like the lung, liver, or kidney, may result in necrosis and wound dehiscence. In addition to this, surgical operations frequently elicit significant health issues associated with haemorrhage. Minimally invasive procedures encounter notable challenges associated with bleeding, such as the potential impairment of eyesight during ocular treatments. Consequently, surgical sealants, possessing the twin capability of hemostasis and wound closure, have emerged as a crucial element in the surgical repertoire for the purpose of controlling persistent bleeding. (Chiara et al., 2018). Currently, within the domain of sutureless surgical techniques, a diverse array of protein-based adhesives (such as fibrin, collagen, and gelatin), polysaccharide-based adhesives (including chitosan and alginate), as well as cyanoacrylate-based adhesives, are employed for a multitude of clinical wound closure purposes (Baghdasarian et al., 2022).

Sutures are the primary surgical wound closure method, but they can cause foreign body reactions and atypical collagen accumulation, leading to hypertrophic scars. The development of suture materials that can effectively suppress inflammation and minimize scar formation is of paramount clinical importance. The S@LC@CGTP suture material was developed which was a combination of a conventional 3-0 PPDO (poly(p-dioxanone)) suture and two additional layers, showed promising sustained-drug release characteristics in vitro and in vivo. The drug-loaded layer contained curcumin, while the electroactive layer was made of oligochitosan-gelatin/tannic acid/polypyrrole. Collectively, S@LC@CGTP suture material possesses significant promise in promoting ideal, scar-free wound healing following surgical incisions (Han et al., 2023).

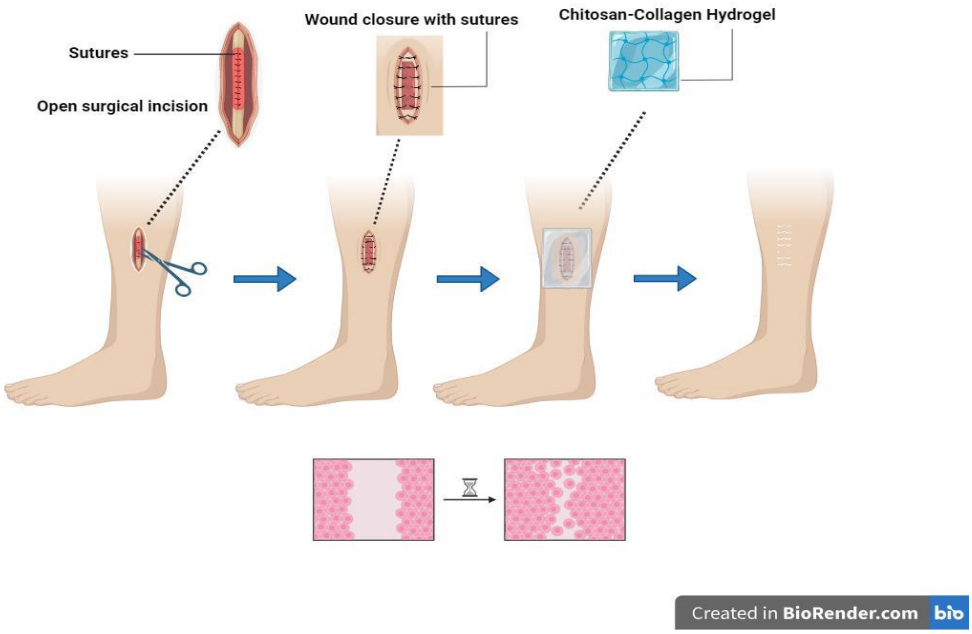

Fig.16: Chitosan-collagen hydrogels accelerate surgical wound closure and healing

Composite hydrogels referred to as Gel-ZBG consisting of ZBG (zinc doped bioactive glass), SCS (succinyl chitosan), and OAL (oxidized alginate) have been developed. These were designed specifically for use as wound dressings, with the aim of promoting faster wound closure in **Fig. 16**. The incorporation of Schiff-based connections into composite hydrogels has been implemented to create a moist microenvironment that promotes cell growth at wound sites. The composite hydrogels were shown to possess remarkable antibacterial capabilities, as

evidenced by the in vitro antibacterial assays. The presence of Si4+ and Ca2+ ions is crucial in promoting the secretion of beneficial factors by fibroblasts, hence facilitating the processes of angiogenesis and wound closure (Jiangying Zhu et al., 2019).

The primary hazards contributing to casualties in catastrophes are extensive bleeding and wound infection resulting from tissue trauma, which demand first-aid provisions that can successfully facilitate wound closure, as well as efficiently manage haemorrhage and infection. The hemostatic and antibacterial properties of current tissue adhesives are often limited. A liquid bandage (LBA) called NB-CMC/CMC hydrogel is an in-situ imine crosslinking-based photoresponsive chitosan hydrogel. The modified carboxymethyl chitosan (CMC) demonstrates enhanced tissue adhesive performance. Furthermore, it demonstrates favorable biocompatibility, biodegradability, and the potential to augment the wound healing process and for wound closure (Ma et al., 2020).

Suture lines have been used for wound healing for millennia, with polysaccharides like celluloses, starches, and glycogens being common. Cotton is a popular suture material, but regenerated celluloses and hydrogel polysaccharide materials like chitosan and alginate are also used in modern medical practice (Peng et al., 2020). The experimental findings indicate that the groups treated with the hydrogels exhibited accelerated wound recovery and earlier healing compared to the control groups. This advocates a considerable advancement in the use of hydrogels for wound repair and closure of wounds (Pan et al., 2019).

VII. CHALLENGES IN THE DEVELOPMENT AND USE OF INNOVATIONS FOR THE BETTERMENT

A hydrogel must meet application-specific design criteria to suitably treat a medical condition. Broadly, these design criteria can be defined as either physical, chemical, or biological. Despite the success of hydrogel-based delivery systems, key technological challenges including chemistry, manufacturing and controls, defined regulatory guidelines, and practical adaptability remain as major roadblocks in their successful clinical translation (Mandal et al., 2020). Since hydrogel fabrication is complex and varies between hydrogel systems, the development costs through clinical translation range in estimation from $50 million up to $800 million (Li & Mooney, 2016). Despite being a well-known biopolymer with various biomedical applications, chitosan also has some limitations such as small specific surface area and void fraction that should be overcome (Esquerdo et al., 2014). While collagen could control drug release from hydrogels and could induce cell growth (Ghasemiyeh & Mohammadi-Samani, 2019).

Chemical and physical crosslinking methods using glutaraldehyde (GA) and ammonium hydroxide (AH), respectively, were utilized to prepare chitosan (CS) and chitosan/collagen (CS-Co) hydrogels; these materials were then subjected to freeze-drying process to obtain 3D porous scaffolds. Physically crosslinked scaffolds exhibited a homogeneous morphology with higher pore size and interconnectivity in comparison to other prepared scaffolds; also, these samples, showed a good biocompatibility. Scaffolds derived from hydrogels treated with acetone (AC) showed shrinkage, smaller pore size and higher degradation rates; finally, materials chemically crosslinked with glutaraldehyde presented cytotoxicity and exhibited a heterogeneous morphology (Reyna-Urrutia et al., 2019).

The collagen–chitosan material was more resistant to enzymatic degradation and was better able to maintain its form over time. Ideally, the degradation rate of the biomaterial matches the regeneration rate of the host tissue and the collagen–chitosan degradation rate can be regulated by adjusting the ratio of collagen to chitosan. The ability to control degradation and structural integrity should prevent a sudden loss of mechanical properties that may occur from materials with more rapid degradation rates. It is likely that the addition of chitosan conferred these improved physical properties, at least in part, through the provision of additional amino groups, which would serve to increase the crosslinking density and reinforce the hydrogel (Deng et al., 2010).

Graphene oxide (GO) a nanostructure with high surface area and high surface functional groups enhances the biological properties of collagen hydrogel scaffold for neural stem/precursor cells (NS/PCs). Addition of 1%–1.5% GO to collagen hydrogel (by weight of collagen) provided a hydrogel in which nNS/PCs could survive and migrate more in comparison with control (polyl-lysine coated well) and pure collagen. Also, GO modulates elasticity of collagen hydrogels and makes it favorable for neural stem cells (Rezaei et al., 2021). A composite

hydrogel was developed consisting of photocrosslinkable methacrylated glycol chitosan (MeGC) and semi-interpenetrating collagen (Col) with a riboflavin photoinitiator under blue light. The incorporation of Col in MeGC hydrogels enhanced the compressive modulus and slowed the degradation rate of the hydrogels. MeGC–Col composite hydrogels significantly enhanced cellular attachment, spreading, proliferation and osteogenic differentiation of mouse bone marrow stromal cells (BMSCs) seeded on the hydrogels compared with pure MeGC hydrogels, as observed by upregulated alkaline phosphatase (ALP) activity as well as increased mineralization (Arakawa et al., 2017).

VIII. CONCLUSION

In conclusion, temperature- and pH-sensitive chitosan-collagen hydrogels have a variety of applications in wound healing along with effective antimicrobial potential. The combined beneficial effects of chitosan and collagen in composite hydrogels have enabled health professionals to treat wounds that were hard to heal in the past with traditional dressings. Chitosan helps in the release of antimicrobial agents in response to changes in temperature and pH, while collagen plays a role in wound healing by promoting tissue regeneration. Chitosan-collagen hydrogels stimulate advanced wound healing compared to commercial dressings, proving their potential as dressings for full-thickness skin wound healing. Their temperature and pH sensitivity make them able to perform their activity in the desired manner. The composite chitosan-collagen hydrogels have improved hydrophilicity, which prevents the immediate dissolution of the hydrogel in an aqueous solution. Combining chitosan and collagen results in hydrogels with higher mechanical strength in comparison to mere chitosan or collagen hydrogels. A moist environment appropriate for wound healing is efficiently maintained by chitosan-collagen hydrogels, along with a slow degradation rate. These novel biomaterials have not only the exceptional attributes of chitosan and collagen but also the ability to respond to temperature and pH changes, which makes them important defense equipment against infections and promotes wound healing. The biocompatibility of temperature- and pH-sensitive chitosan-collagen hydrogels make them an innovative solution for healing chronic wounds and controlling infections. Further research in this domain can unlock more opportunities for cheaper production of these biomaterials for the treatment of a variety of health complications.

REFRENCES

[1] Agarwal, V., Toshniwal, P., Smith, N. E., Smith, N. M., Li, B., Clemons, T. D., Byrne, L. T., Kakulas, F., Wood, F. M., & Fear, M. (2016). Enhancing the efficacy of cation-independent mannose 6-phosphate receptor inhibitors by intracellular delivery. *Chemical communications*, *52*(2), 327-330.

[2] Ahmad, Z., Salman, S., Khan, S. A., Amin, A., Rahman, Z. U., Al-Ghamdi, Y. O., Akhtar, K., Bakhsh, E. M., & Khan, S. B. (2022). Versatility of hydrogels: from synthetic strategies, classification, and properties to biomedical applications. *Gels*, *8*(3), 167.

[3] Ahmadian, Z., Correia, A., Hasany, M., Figueiredo, P., Dobakhti, F., Eskandari, M. R., Hosseini, S. H., Abiri, R., Khorshid, S., & Hirvonen, J. (2021). A hydrogen-bonded extracellular matrix-mimicking bactericidal hydrogel with radical scavenging and hemostatic function for pH-responsive wound healing acceleration. *Advanced Healthcare Materials*, *10*(3), 2001122.

[4] Ahmed, S., & Ikram, S. (2016). Chitosan based scaffolds and their applications in wound healing. *Achievements in the life sciences*, *10*(1), 27-37.

[5] Alagha, A., Nourallah, A., & Hariri, S. (2020). Characterization of dexamethasone loaded collagen-chitosan sponge and in vitro release study. *Journal of Drug Delivery Science and Technology*, *55*, 101449. https://doi.org/https://doi.org/10.1016/j.jddst.2019.101449

[6] Alberti, T. B., Coelho, D. S., de Prá, M., Maraschin, M., & Veleirinho, B. (2020). Electrospun PVA nanoscaffolds associated with propolis nanoparticles with wound healing activity. *Journal of Materials Science*, *55*(23), 9712-9727.

[7] Aleem, A. R., Shahzadi, L., Tehseen, S., Alvi, F., Chaudhry, A. A., Rehman, I. u., & Yar, M. (2019). Amino acids loaded chitosan/collagen based new membranes stimulate angiogenesis in chorioallantoic membrane assay. *International Journal of Biological Macromolecules*, *140*, 401-406. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.08.095

[8] Ali, A., & Ahmed, S. (2018). A review on chitosan and its nanocomposites in drug delivery. *International Journal of Biological Macromolecules*, *109*, 273-286. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2017.12.078

[9] Ali, A. F., Ahmed, M. M., El-Kady, A. M., Abd El-Hady, B. M., & Ibrahim, A. M. (2021). Synthesis of gelatin-agarose scaffold for controlled antibiotic delivery and its modification by glass nanoparticles addition as a potential osteomyelitis treatment. *Silicon*, *13*, 2011-2028.

[10] Almajed, A., Lemboye, K., & Moghal, A. A. B. (2022). A critical review on the feasibility of synthetic polymers inclusion in enhancing the geotechnical behavior of soils. *Polymers*, *14*(22), 5004.

[11] Alven, S., & Aderibigbe, B. A. (2020). Chitosan and Cellulose-Based Hydrogels for Wound Management. *International Journal of Molecular Sciences*, *21*(24), 9656. https://www.mdpi.com/1422-0067/21/24/9656

[12] Alven, S., & Aderibigbe, B. A. (2021). Hyaluronic acid-based scaffolds as potential bioactive wound dressings. *Polymers*, *13*(13), 2102.

[13] Arakawa, C., Ng, R., Tan, S., Kim, S., Wu, B., & Lee, M. (2017). Photopolymerizable chitosan–collagen hydrogels for bone tissue engineering. *Journal of Tissue Engineering and Regenerative Medicine*, *11*(1), 164-174. https://doi.org/https://doi.org/10.1002/term.1896

[14] Arbia, W., Arbia, L., Adour, L., & Amrane, A. (2013). Chitin extraction from crustacean shells using biological methods–a review. *Food Technology and Biotechnology*, *51*(1), 12-25.

[15] Ata, S., Rasool, A., Islam, A., Bibi, I., Rizwan, M., Azeem, M. K., & Iqbal, M. (2020). Loading of Cefixime to pH sensitive chitosan based hydrogel and investigation of controlled release kinetics. *International Journal of Biological Macromolecules*, *155*, 1236-1244.

[16] Atalay, S., Jarocka-Karpowicz, I., & Skrzydlewska, E. (2019). Antioxidative and anti-inflammatory properties of cannabidiol. *Antioxidants*, *9*(1), 21.

[17] Azaza, Y. B., van der lee, A., Li, S., Nasri, M., & Nasri, R. (2023). Chitosan/collagen-based hydrogels for sustainable development: Phycocyanin controlled release. *Sustainable Chemistry and Pharmacy*, *31*, 100905. https://doi.org/https://doi.org/10.1016/j.scp.2022.100905

[18] Baghaie, S., Khorasani, M. T., Zarrabi, A., & Moshtaghian, J. (2017). Wound healing properties of PVA/starch/chitosan hydrogel membranes with nano Zinc oxide as antibacterial wound dressing material. *Journal of Biomaterials Science, Polymer Edition*, *28*(18), 2220-2241.

[19] Baghdasarian, S., Saleh, B., Baidya, A., Kim, H., Ghovvati, M., Sani, E. S., Haghniaz, R., Madhu, S., Kanelli, M., Noshadi, I., & Annabi, N. (2022). Engineering a naturally derived hemostatic sealant for sealing internal organs. *Materials Today Bio*, *13*, 100199. https://doi.org/https://doi.org/10.1016/j.mtbio.2021.100199

[20] Banerjee, S., Szepes, M., Dibbert, N., Rios-Camacho, J.-C., Kirschning, A., Gruh, I., & Dräger, G. (2021). Dextran-based scaffolds for in-situ hydrogelation: Use for next generation of bioartificial cardiac tissues. *Carbohydrate Polymers*, *262*, 117924.

[21] Barroso, T., Viveiros, R., Casimiro, T., & Aguiar-Ricardo, A. (2014). Development of dual-responsive chitosan–collagen scaffolds for pulsatile release of bioactive molecules. *The Journal of Supercritical Fluids*, *94*, 102-112.

[22] Bautista, J., Jover, M., Gutierrez, J., Corpas, R., Cremades, O., Fontiveros, E., Iglesias, F., & Vega, J. (2001). Preparation of crayfish chitin by in situ lactic acid production. *Process biochemistry*, *37*(3), 229-234.

[23] Bax, D. V., Davidenko, N., Hamaia, S. W., Farndale, R. W., Best, S. M., & Cameron, R. E. (2019). Impact of UV-and carbodiimide-based crosslinking on the integrin-binding properties of collagen-based materials. *Acta biomaterialia*, *100*, 280-291.

[24] Berger, J., Reist, M., Mayer, J., Felt, O., Peppas, N., & Gurny, R. (2004). Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *European journal of pharmaceutics and biopharmaceutics*, *57*(1), 19-34.

[25] Bhattarai, N., Gunn, J., & Zhang, M. (2010). Chitosan-based hydrogels for controlled, localized drug delivery. *Advanced drug delivery reviews*, *62*(1), 83-99.

[26] Bolke, L., Schlippe, G., Gerß, J., & Voss, W. (2019). A collagen supplement improves skin hydration, elasticity, roughness, and density: Results of a randomized, placebo-controlled, blind study. *Nutrients*, *11*(10), 2494.

[27] Brzeska, J., Tercjak, A., Sikorska, W., Kowalczuk, M., & Rutkowska, M. (2019). Morphology and physicochemical properties of branched polyurethane/biopolymer blends. *Polymers*, *12*(1), 16.

[28] Burkatovskaya, M., Tegos, G. P., Swietlik, E., Demidova, T. N., Castano, A. P., & Hamblin, M. R. (2006). Use of chitosan bandage to prevent fatal infections developing from highly contaminated wounds in mice. *Biomaterials*, *27*(22), 4157-4164.

[29] Çakmak, A., Çirpanli, Y., Bilensoy, E., Yorganci, K., Çaliş, S., Saribaş, Z., & Kaynaroğlu, V. (2009). Antibacterial activity of triclosan chitosan coated graft on hernia graft infection model. *International journal of pharmaceutics*, *381*(2), 214-219.

[30] Cao, J., Wang, P., Liu, Y., Zhu, C., & Fan, D. (2020). Double crosslinked HLC-CCS hydrogel tissue engineering scaffold for skin wound healing. *International Journal of Biological Macromolecules*, *155*, 625-635.

[31] Cao, J., You, J., Zhang, L., & Zhou, J. (2018). Homogeneous synthesis and characterization of chitosan ethers prepared in aqueous alkali/urea solutions. *Carbohydrate Polymers*, *185*, 138-144.

[32] Cardoso-Daodu, I. M., Ilomuanya, M. O., & Azubuike, C. P. (2022). Development of curcumin-loaded liposomes in lysine–collagen hydrogel for surgical wound healing. *Beni-Suef University Journal of Basic and Applied Sciences*, *11*(1), 100. https://doi.org/10.1186/s43088-022-00284-2

[33] Carvalho, D. N., Gonçalves, C., Oliveira, J. M., Williams, D. S., Mearns-Spragg, A., Reis, R. L., & Silva, T. H. (2022). A design of experiments (DoE) approach to optimize cryogel manufacturing for tissue engineering applications. *Polymers*, *14*(10), 2026.

[34] Catoira, M. C., Fusaro, L., Di Francesco, D., Ramella, M., & Boccafoschi, F. (2019). Overview of natural hydrogels for regenerative medicine applications. *Journal of Materials Science: Materials in Medicine*, *30*(10), 115. https://doi.org/10.1007/s10856-019-6318-7

[35] Chang, S.-W., Shefelbine, S. J., & Buehler, M. J. (2012). Structural and mechanical differences between collagen homo-and heterotrimers: relevance for the molecular origin of brittle bone disease. *Biophysical journal*, *102*(3), 640-648.

[36] Chaves, L. L., Silveri, A., Vieira, A. C. C., Ferreira, D., Cristiano, M. C., Paolino, D., Di Marzio, L., Lima, S. C., Reis, S., Sarmento, B., & Celia, C. (2019). pH-responsive chitosan based hydrogels affect the release of dapsone: Design, set-up, and physicochemical characterization. *International Journal of Biological Macromolecules*, *133*, 1268-1279.

https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.04.178

[37] Chen, G., Ren, J., Deng, Y., Wu, X., Huang, J., Wang, G., Zhao, Y., & Li, J. (2017). An injectable, wound-adapting, self-healing hydrogel for fibroblast growth factor 2 delivery system in tissue repair applications. *Journal of biomedical nanotechnology*, *13*(12), 1660-1672.

[38] Chen, H., Xing, X., Tan, H., Jia, Y., Zhou, T., Chen, Y., Ling, Z., & Hu, X. (2017). Covalently antibacterial alginate-chitosan hydrogel dressing integrated gelatin microspheres containing tetracycline hydrochloride for wound healing. *Materials Science and Engineering: C*, *70*, 287-295.

[39] Cheng, S., Wang, W., Li, Y., Gao, G., Zhang, K., Zhou, J., & Wu, Z. (2019). Cross-linking and film-forming properties of transglutaminase-modified collagen fibers tailored by denaturation temperature. *Food chemistry*, *271*, 527-535.

[40] Cheng, X., Shao, Z., Li, C., Yu, L., Raja, M. A., & Liu, C. (2017). Isolation, characterization and evaluation of collagen from jellyfish Rhopilema esculentum Kishinouye for use in hemostatic applications. *PloS one*, *12*(1), e0169731.

[41] Chiara, O., Cimbanassi, S., Bellanova, G., Chiarugi, M., Mingoli, A., Olivero, G., Ribaldi, S., Tugnoli, G., Basilicò, S., & Bindi, F. (2018). A systematic review on the use of topical hemostats in trauma and emergency surgery. *BMC surgery*, *18*(1), 1-20.

[42] Cho, J., Heuzey, M.-C., Bégin, A., & Carreau, P. J. (2005). Physical Gelation of Chitosan in the Presence of β-Glycerophosphate: The Effect of Temperature. *Biomacromolecules*, *6*(6), 3267-3275. https://doi.org/10.1021/bm050313s

[43] Chuysinuan, P., Thanyacharoen, T., Thongchai, K., Techasakul, S., & Ummartyotin, S. (2020). Preparation of chitosan/hydrolyzed collagen/hyaluronic acid based hydrogel composite with caffeic acid addition. *International Journal of Biological Macromolecules*, *162*, 1937-1943. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.08.139

[44] Croisier, F., & Jérôme, C. (2013). Chitosan-based biomaterials for tissue engineering. *European Polymer Journal*, *49*(4), 780-792.

[45] Cui, F., Li, G., Huang, J., Zhang, J., Lu, M., Lu, W., Huan, J., & Huang, Q. (2011). Development of chitosan-collagen hydrogel incorporated with lysostaphin (CCHL) burn dressing with anti-methicillin-resistant Staphylococcus aureus and promotion wound healing properties. *Drug delivery*, *18*(3), 173-180.

[46] Dai, T., Tegos, G. P., Burkatovskaya, M., Castano, A. P., & Hamblin, M. R. (2009). Chitosan acetate bandage as a topical antimicrobial dressing for infected burns. *Antimicrobial agents and chemotherapy*, *53*(2), 393-400.

[47] Deng, C., Zhang, P., Vulesevic, B., Kuraitis, D., Li, F., Yang, A. F., Griffith, M., Ruel, M., & Suuronen, E. J. (2010). A collagen–chitosan hydrogel for endothelial differentiation and angiogenesis. *Tissue Engineering Part A*, *16*(10), 3099-3109.

[48] Díaz-Montes, E. (2021). Dextran: sources, structures, and properties. *Polysaccharides*, *2*(3), 554-565.

[49] Djavid, G. E., Tabaie, S. M., Tajali, S. B., Totounchi, M., Farhoud, A., Fateh, M., Ghafghazi, M., Koosha, M., & Taghizadeh, S. (2020). Application of a collagen matrix dressing on a neuropathic diabetic foot ulcer: a randomised control trial. *Journal of Wound Care*, *29*(Sup3), S13-S18. https://doi.org/10.12968/jowc.2020.29.Sup3.S13

[50] Dong, C., & Lv, Y. (2016). Application of collagen scaffold in tissue engineering: recent advances and new perspectives. *Polymers*, *8*(2), 42.

[51] Dong, Y., Hassan, W. U., Kennedy, R., Greiser, U., Pandit, A., Garcia, Y., & Wang, W. (2014). Performance of an in situ formed bioactive hydrogel dressing from a PEG-based hyperbranched multifunctional copolymer. *Acta biomaterialia*, *10*(5), 2076-2085.

[52] Duan, R., Zhang, J., Du, X., Yao, X., & Konno, K. (2009). Properties of collagen from skin, scale and bone of carp (Cyprinus carpio). *Food chemistry*, *112*(3), 702-706.

[53] Duceac, I. A., Lobiuc, A., Coseri, S., & Verestiuc, L. (2019, 21-23 Nov. 2019). Tunable Hydrogels based on Chitosan, Collagen and Poly(Acrylic Acid) for Regenerative Medicine. 2019 E-Health and Bioengineering Conference (EHB),

[54] Ertl, P., Altmann, E., & McKenna, J. M. (2020). The most common functional groups in bioactive molecules and how their popularity has evolved over time. *Journal of medicinal chemistry*, *63*(15), 8408-8418.

[55] Esquerdo, V. M., Cadaval Jr, T., Dotto, G., & Pinto, L. (2014). Chitosan scaffold as an alternative adsorbent for the removal of hazardous food dyes from aqueous solutions. *Journal of colloid and interface science*, *424*, 7-15.

[56] Ezati, P., & Rhim, J.-W. (2020). pH-responsive chitosan-based film incorporated with alizarin for intelligent packaging applications. *Food Hydrocolloids*, *102*, 105629.

[57] Fadeeva, I. V., Trofimchuk, E. S., Forysenkova, A. A., Ahmed, A. I., Gnezdilov, O. I., Davydova, G. A., Kozlova, S. G., Antoniac, A., & Rau, J. V. (2021). Composite polyvinylpyrrolidone–sodium alginate—Hydroxyapatite hydrogel films for bone repair and wound dressings applications. *Polymers*, *13*(22), 3989.

[58] Fan, Z., Liu, B., Wang, J., Zhang, S., Lin, Q., Gong, P., Ma, L., & Yang, S. (2014). A novel wound dressing based on Ag/graphene polymer hydrogel: effectively kill bacteria and accelerate wound healing. *Advanced Functional Materials*, *24*(25), 3933-3943.

[59] Fatemi, M. J., Garahgheshlagh, S. N., Ghadimi, T., Jamili, S., Nourani, M. R., Sharifi, A. M., Saberi, M., Amini, N., Sarmadi, V. H., & Yazdi-Amirkhiz, S. Y. (2021). Investigating the Impact of Collagen-Chitosan Derived from Scomberomorus Guttatus and Shrimp Skin on Second-Degree Burn in Rats Model. *Regenerative Therapy*, *18*, 12-20. https://doi.org/https://doi.org/10.1016/j.reth.2021.03.001

[60] Fathi, M., Alami-Milani, M., Geranmayeh, M. H., Barar, J., Erfan-Niya, H., & Omidi, Y. (2019). Dual thermo-and pH-sensitive injectable hydrogels of chitosan/(poly(N-isopropylacrylamide-co-itaconic acid)) for doxorubicin delivery in breast cancer. *International Journal of Biological Macromolecules*, *128*, 957-964.

https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.01.122

[61] Felician, F. F., Xia, C., Qi, W., & Xu, H. (2018). Collagen from marine biological sources and medical applications. *Chemistry & biodiversity*, *15*(5), e1700557.

[62] Feng, X., Zhang, X., Li, S., Zheng, Y., Shi, X., Li, F., Guo, S., & Yang, J. (2020). Preparation of aminated fish scale collagen and oxidized sodium alginate hybrid hydrogel for enhanced full-thickness wound healing. *International Journal of Biological Macromolecules*, *164*, 626-637.

[63] Ferrario, C., Rusconi, F., Pulaj, A., Macchi, R., Landini, P., Paroni, M., Colombo, G., Martinello, T., Melotti, L., & Gomiero, C. (2020). From food waste to innovative biomaterial: Sea urchin-derived collagen for applications in skin regenerative medicine. *Marine Drugs*, *18*(8), 414.

[64] Fidalgo, C., Rodrigues, M., Peixoto, T., Lobato, J., Santos, J., & Lopes, M. (2018). Development of asymmetric resorbable membranes for guided bone and surrounding tissue regeneration. *Journal of Biomedical Materials Research Part A*, *106*(8), 2141-2150.

[65] Forero, J. C., Roa, E., Reyes, J. G., Acevedo, C., & Osses, N. (2017). Development of useful biomaterial for bone tissue engineering by incorporating nano-copper-zinc alloy (nCuZn) in chitosan/gelatin/nano-hydroxyapatite (Ch/G/nHAp) scaffold. *Materials*, *10*(10), 1177.

[66] Frayssinet, A., Petta, D., Illoul, C., Haye, B., Markitantova, A., Eglin, D., Mosser, G., D'este, M., & Hélary, C. (2020). Extracellular matrix-mimetic composite hydrogels of cross-linked hyaluronan and fibrillar collagen with tunable properties and ultrastructure. *Carbohydrate Polymers*, *236*, 116042.

[67] Freytes, D. O., Martin, J., Velankar, S. S., Lee, A. S., & Badylak, S. F. (2008). Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. *Biomaterials*, *29*(11), 1630-1637.

[68] Fu, G., Chen, Y., Cui, Z., Li, Y., Zhou, W., Xin, S., Tang, Y., & Goodenough, J. B. (2016). Novel hydrogel-derived bifunctional oxygen electrocatalyst for rechargeable air cathodes. *Nano letters*, *16*(10), 6516-6522.

[69] Garcia-Orue, I., Santos-Vizcaino, E., Etxabide, A., Uranga, J., Bayat, A., Guerrero, P., Igartua, M., de la Caba, K., & Hernandez, R. M. (2019). Development of bioinspired gelatin and gelatin/chitosan bilayer hydrofilms for wound healing. *Pharmaceutics*, *11*(7), 314.

[70] Garnica-Palafox, I., & Sánchez-Arévalo, F. (2016). Influence of natural and synthetic crosslinking reagents on the structural and mechanical properties of chitosan-based hybrid hydrogels. *Carbohydrate Polymers*, *151*, 1073-1081.

[71] Gasperini, L., Mano, J. F., & Reis, R. L. (2014). Natural polymers for the microencapsulation of cells. *Journal of the royal society Interface*, *11*(100), 20140817.

[72] Gaware, S. A., Rokade, K. A., & Kale, S. (2019). Silica-chitosan nanocomposite mediated pH-sensitive drug delivery. *Journal of Drug Delivery Science and Technology*, *49*, 345-351.

[73] Ge, B., Wang, H., Li, J., Liu, H., Yin, Y., Zhang, N., & Qin, S. (2020). Comprehensive assessment of Nile tilapia skin (Oreochromis niloticus) collagen hydrogels for wound dressings. *Marine Drugs*, *18*(4), 178.

[74] Geng, H., Zhang, P., Liu, L., Shangguan, Y., Cheng, X., Liu, H., Zhao, Y., Hao, J., Li, W., & Cui, J. (2022). Convergent architecting of multifunction-in-one hydrogels as wound dressings for surgical anti-infections. *Materials Today Chemistry*, *25*, 100968. https://doi.org/https://doi.org/10.1016/j.mtchem.2022.100968

[75] Ghasemiyeh, P., & Mohammadi-Samani, S. (2019). Hydrogels as drug delivery systems; pros and cons. *Trends in Pharmaceutical Sciences*, *5*(1), 7-24.

[76] Gilarska, A., Lewandowska-Łańcucka, J., Guzdek-Zając, K., Karewicz, A., Horak, W., Lach, R., Wójcik, K., & Nowakowska, M. (2020). Bioactive yet antimicrobial structurally stable collagen/chitosan/lysine functionalized hyaluronic acid – based injectable hydrogels for potential bone tissue engineering applications. *International Journal of Biological Macromolecules*, *155*, 938-950. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2019.11.052

[77] Golieskardi, M., Satgunam, M., Ragurajan, D., Hoque, M. E., & Ng, A. M. H. (2020). Microstructural, Tribological, and Degradation Properties of Al 2 O 3-and CeO 2-Doped 3 mol.% Yttria-Stabilized Zirconia Bioceramic for Biomedical Applications. *Journal of Materials Engineering and Performance*, *29*, 2890-2897.

[78] Gomez-Aparicio, L. S., Bernáldez-Sarabia, J., Camacho-Villegas, T. A., Lugo-Fabres, P. H., Díaz-Martínez, N. E., Padilla-Camberos, E., Licea-Navarro, A., & Castro-Ceseña, A. B. (2021). Improvement of the wound healing properties of hydrogels with N-acetylcysteine through their modification with methacrylate-containing polymers. *Biomaterials Science*, *9*(3), 726-744.

[79] Grabska-Zielińska, S., Pin, J. M., Kaczmarek-Szczepańska, B., Olewnik-Kruszkowska, E., Sionkowska, A., Monteiro, F. J., Steinbrink, K., & Kleszczyński, K. (2022). Scaffolds Loaded with Dialdehyde Chitosan and Collagen—Their Physico-Chemical Properties and Biological Assessment. *Polymers*, *14*(9), 1818.

[80] Gu, L., Shan, T., Ma, Y.-x., Tay, F. R., & Niu, L. (2019). Novel biomedical applications of crosslinked collagen. *Trends in biotechnology*, *37*(5), 464-491.

[81] Güiza-Argüello, V. R., Solarte-David, V. A., Pinzón-Mora, A. V., Ávila-Quiroga, J. E., & Becerra-Bayona, S. M. (2022). Current Advances in the Development of Hydrogel-Based Wound Dressings for Diabetic Foot Ulcer Treatment. *Polymers*, *14*(14), 2764. https://www.mdpi.com/2073-4360/14/14/2764

[82] Gunes, O. C., Kara, A., Baysan, G., Bugra Husemoglu, R., Akokay, P., Ziylan Albayrak, A., Ergur, B. U., & Havitcioglu, H. (2022). Fabrication of 3D Printed poly(lactic acid) strut and wet-electrospun cellulose nano fiber reinforced chitosan-collagen hydrogel composite scaffolds for meniscus tissue engineering. *Journal of Biomaterials Applications*, *37*(4), 683-697. https://doi.org/10.1177/08853282221109339

[83] Gunes, O. C., & Ziylan Albayrak, A. (2021). Antibacterial Polypeptide nisin containing cotton modified hydrogel composite wound dressings. *Polymer Bulletin*, *78*, 6409-6428.

[84] Guo, S. Z., Gosselin, F., Guerin, N., Lanouette, A. M., Heuzey, M. C., & Therriault, D. (2013). Solvent-cast three-dimensional printing of multifunctional microsystems. *Small*, *9*(24), 4118-4122.

[85] Gupta, A., & Kim, B. S. (2019). Shape memory polyurethane biocomposites based on toughened polycaprolactone promoted by nano-chitosan. *Nanomaterials*, *9*(2), 225.

[86] Gupta, A., Kowalczuk, M., Heaselgrave, W., Britland, S. T., Martin, C., & Radecka, I. (2019). The production and application of hydrogels for wound management: A review. *European Polymer Journal*, *111*, 134-151. https://doi.org/https://doi.org/10.1016/j.eurpolymj.2018.12.019

[87] Haaparanta, A.-M., Järvinen, E., Cengiz, I. F., Ellä, V., Kokkonen, H. T., Kiviranta, I., & Kellomäki, M. (2014). Preparation and characterization of collagen/PLA, chitosan/PLA, and collagen/chitosan/PLA hybrid scaffolds for cartilage tissue engineering. *Journal of Materials Science: Materials in Medicine*, *25*, 1129-1136.

[88] Han, H., Tang, L., Li, Y., Li, Y., Bi, M., Wang, J., Wang, F., Wang, L., & Mao, J. (2023). A multifunctional surgical suture with electroactivity assisted by oligochitosan/gelatin-tannic acid for promoting skin wound healing and controlling scar proliferation. *Carbohydrate Polymers*, *320*, 121236. https://doi.org/https://doi.org/10.1016/j.carbpol.2023.121236

[89] Han, W., & Wang, S. (2023). Advances in Hemostatic Hydrogels That Can Adhere to Wet Surfaces. *Gels*, *9*(1), 2. https://www.mdpi.com/2310-2861/9/1/2

[90] Hemmatgir, F., Koupaei, N., & Poorazizi, E. (2022). Characterization of a novel semi-interpenetrating hydrogel network fabricated by polyethylene glycol diacrylate/polyvinyl alcohol/tragacanth gum as a wound dressing. *Burns*, *48*(1), 146-155.

[91] Hernández-Martínez, A. R., Molina, G. A., Jiménez-Hernández, L. F., Oskam, A. H., Fonseca, G., & Estevez, M. (2017). Evaluation of inulin replacing chitosan in a polyurethane/polysaccharide material for Pb2+ removal. *Molecules*, *22*(12), 2093.

[92] Hong, Y., Zhou, F., Hua, Y., Zhang, X., Ni, C., Pan, D., Zhang, Y., Jiang, D., Yang, L., & Lin, Q. (2019). A strongly adhesive hemostatic hydrogel for the repair of arterial and heart bleeds. *Nature communications*, *10*(1), 2060.

[93] Huang, H., Qi, X., Chen, Y., & Wu, Z. (2019). Thermo-sensitive hydrogels for delivering biotherapeutic molecules: A review. *Saudi Pharmaceutical Journal*, *27*(7), 990-999.

[94] Irastorza-Lorenzo, A., Sánchez-Porras, D., Ortiz-Arrabal, O., de Frutos, M. J., Esteban, E., Fernández, J., Janer, A., Campos, A., Campos, F., & Alaminos, M. (2021). Evaluation of marine agarose biomaterials for tissue engineering applications. *International Journal of Molecular Sciences*, *22*(4), 1923.

[95] Jiang, H., Zheng, M., Liu, X., Zhang, S., Wang, X., Chen, Y., Hou, M., & Zhu, J. (2019). Feasibility study of tissue transglutaminase for self-catalytic cross-linking of self-assembled collagen fibril hydrogel and its promising application in wound healing promotion. *ACS Omega*, *4*(7), 12606-12615.

[96] Jimoh, T. O., Ogunmoyole, T., Aladejana, E. A., & Kade, I. J. (2016). Antioxidant potentials of tannic acid on lipid peroxidation induced by several pro-oxidants in cerebral and hepatic lipids. *International Journal of Ethnopharmacology*, *2 (1)*, 014-020.

[97] Jin, Y., Ling, P.-X., He, Y.-L., & Zhang, T.-M. (2007). Effects of chitosan and heparin on early extension of burns. *Burns*, *33*(8), 1027-1031.

[98] Kalai Selvan, N., Shanmugarajan, T. S., & Uppuluri, V. N. V. A. (2020). Hydrogel based scaffolding polymeric biomaterials: Approaches towards skin tissue regeneration. *Journal of Drug Delivery Science and Technology*, *55*, 101456. https://doi.org/https://doi.org/10.1016/j.jddst.2019.101456

[99] Kamali, A., & Shamloo, A. (2020). Fabrication and evaluation of a bilayer hydrogel-electrospinning scaffold prepared by the freeze-gelation method. *Journal of Biomechanics*, *98*, 109466. https://doi.org/https://doi.org/10.1016/j.jbiomech.2019.109466

[100] Kamoun, E. A., Kenawy, E.-R. S., & Chen, X. (2017). A review on polymeric hydrogel membranes for wound dressing applications: PVA-based hydrogel dressings. *Journal of advanced research*, *8*(3), 217-233.

[101] Kaul, L., Grundmann, C. E., Köll-Weber, M., Löffler, H., Weiz, A., Zannettino, A. C., Richter, K., & Süss, R. (2022). A thermosensitive, chitosan-based hydrogel as delivery system for antibacterial liposomes to surgical site infections. *Pharmaceutics*, *14*(12), 2841.

[102] Kaviani, A., Zebarjad, S. M., Javadpour, S., Ayatollahi, M., & Bazargan-Lari, R. (2019). Fabrication and characterization of low-cost freeze-gelated chitosan/collagen/hydroxyapatite hydrogel nanocomposite scaffold. *International Journal of Polymer Analysis and Characterization*, *24*(3), 191-203. https://doi.org/10.1080/1023666X.2018.1562477

[103] Khor, E., & Lim, L. Y. (2003). Implantable applications of chitin and chitosan. *Biomaterials*, *24*(13), 2339-2349.

[104] Kim, M. Y., & Kim, J. (2017). Chitosan microgels embedded with catalase nanozyme-loaded mesocellular silica foam for glucose-responsive drug delivery. *ACS Biomaterials Science & Engineering*, *3*(4), 572-578.

[105] Kim, Y., Zharkinbekov, Z., Raziyeva, K., Tabyldiyeva, L., Berikova, K., Zhumagul, D., Temirkhanova, K., & Saparov, A. (2023). Chitosan-Based Biomaterials for Tissue Regeneration. *Pharmaceutics*, *15*(3), 807. https://www.mdpi.com/1999-4923/15/3/807

[106] Kisling, A., Lust, R. M., & Katwa, L. C. (2019). What is the role of peptide fragments of collagen I and IV in health and disease? *Life sciences*, *228*, 30-34.

[107] Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T., & Tanaka, M. (2005). Characterisation of acid-

soluble collagen from skin and bone of bigeye snapper (Priacanthus tayenus). *Food chemistry*, *89*(3), 363-372.

[108] Kokabi, M., Sirousazar, M., & Hassan, Z. M. (2007). PVA–clay nanocomposite hydrogels for wound dressing. *European Polymer Journal*, *43*(3), 773-781.

[109] Kumar, V. A., Taylor, N. L., Jalan, A. A., Hwang, L. K., Wang, B. K., & Hartgerink, J. D. (2014). A nanostructured synthetic collagen mimic for hemostasis. *Biomacromolecules*, *15*(4), 1484-1490.

[110] Kurakula, M. (2020). Prospection of recent chitosan biomedical trends: Evidence from patent analysis (2009–2020). *International Journal of Biological Macromolecules*, *165*, 1924-1938.

[111] Ladet, S., David, L., & Domard, A. (2008). Multi-membrane hydrogels. *Nature*, *452*(7183), 76-79.

[112] Lapi, I., Kolliniati, O., Aspevik, T., Deiktakis, E. E., Axarlis, K., Daskalaki, M. G., Dermitzaki, E., Tzardi, M., Kampranis, S. C., & Marsni, Z. E. (2021). Collagen-containing fish sidestream-derived protein hydrolysates support skin repair via chemokine induction. *Marine Drugs*, *19*(7), 396.

[113] Latza, V., Guerette, P. A., Ding, D., Amini, S., Kumar, A., Schmidt, I., Keating, S., Oxman, N., Weaver, J. C., & Fratzl, P. (2015). Multi-scale thermal stability of a hard thermoplastic protein-based material. *Nature communications*, *6*(1), 8313.

[114] Lauto, A., Stoodley, M., Marcel, H., Avolio, A., Sarris, M., McKenzie, G., Sampson, D., & Foster, L. (2007). In vitro and in vivo tissue repair with laser-activated chitosan adhesive. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery*, *39*(1), 19-27.

[115] Lei, H., Zhu, C., & Fan, D. (2020). Optimization of human-like collagen composite polysaccharide hydrogel dressing preparation using response surface for burn repair. *Carbohydrate Polymers*, *239*, 116249.

[116] Lévesque, S. G., Lim, R. M., & Shoichet, M. S. (2005). Macroporous interconnected dextran scaffolds of controlled porosity for tissue-engineering applications. *Biomaterials*, *26*(35), 7436-7446.

[117] Li, J., & Mooney, D. J. (2016). Designing hydrogels for controlled drug delivery. *Nature Reviews Materials*, *1*(12), 1-17.

[118] Li, J., Yu, F., Chen, G., Liu, J., Li, X.-L., Cheng, B., Mo, X.-M., Chen, C., & Pan, J.-F. (2020). Moist-Retaining, Self-Recoverable, Bioadhesive, and Transparent in Situ Forming Hydrogels To Accelerate Wound Healing. *ACS Applied Materials & Interfaces*, *12*(2), 2023-2038. https://doi.org/10.1021/acsami.9b17180

[119] Li, J., Zhai, D., Lv, F., Yu, Q., Ma, H., Yin, J., Yi, Z., Liu, M., Chang, J., & Wu, C. (2016). Preparation of copper-containing bioactive glass/eggshell membrane nanocomposites for improving angiogenesis, antibacterial activity and wound healing. *Acta biomaterialia*, *36*, 254-266.

[120] Li, M., Han, M., Sun, Y., Hua, Y., Chen, G., & Zhang, L. (2019). Oligoarginine mediated collagen/chitosan gel composite for cutaneous wound healing. *International Journal of Biological Macromolecules*, *122*, 1120-1127. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2018.09.061

[121] Li, S., Wang, L., Yu, X., Wang, C., & Wang, Z. (2018). Synthesis and characterization of a novel double cross-linked hydrogel based on Diels-Alder click reaction and coordination bonding. *Materials Science and Engineering: C*, *82*, 299-309.

[122] Li, W., Zhao, X., Huang, T., Ren, Y., Gong, W., Guo, Y., Wang, J., & Tu, Q. (2021). Preparation of sodium hyaluronate/dopamine/AgNPs hydrogel based on the natural eutetic solvent as an antibaterial wound dressing. *International Journal of Biological Macromolecules*, *191*, 60-70.

[123] Li, Y., Zhu, C., Fan, D., Fu, R., Ma, P., Duan, Z., Li, X., Lei, H., & Chi, L. (2019). A Bi-Layer PVA/CMC/PEG Hydrogel with Gradually Changing Pore Sizes for Wound Dressing. *Macromolecular bioscience*, *19*(5), 1800424.

[124] Lim, Y.-S., Ok, Y.-J., Hwang, S.-Y., Kwak, J.-Y., & Yoon, S. (2019). Marine collagen as a promising biomaterial for biomedical applications. *Marine Drugs*, *17*(8), 467.

[125] Lin, X., Feng, Y., He, Y., Ding, S., & Liu, M. (2023). Engineering design of asymmetric halloysite/chitosan/collagen sponge with hydrophobic coating for high-performance hemostasis dressing. *International Journal of Biological Macromolecules*, *237*, 124148. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2023.124148

[126] Liu, H., Li, D., & Guo, S. (2008). Rheological properties of channel catfish (Ictalurus punctaus) gelatine from fish skins preserved by different methods. *LWT-Food Science and Technology*, *41*(8), 1425-1430.

[127] Liu, X., Zheng, C., Luo, X., Wang, X., & Jiang, H. (2019). Recent advances of collagen-based biomaterials: Multi-hierarchical structure, modification and biomedical applications. *Materials Science and Engineering: C*, *99*, 1509-1522.

[128] Liu, Y., Luo, X., Wu, W., Zhang, A., Lu, B., Zhang, T., & Kong, M. (2021). Dual cure (thermal/photo) composite hydrogel derived from chitosan/collagen for in situ 3D bioprinting. *International Journal of Biological Macromolecules*, *182*, 689-700. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2021.04.058

[129] Loureiro, K. C., Barbosa, T. C., Nery, M., Chaud, M. V., da Silva, C. F., Andrade, L. N., Corrêa, C. B., Jaguer, A., Padilha, F. F., & Cardoso, J. C. (2020). Antibacterial activity of chitosan/collagen membranes containing red propolis extract. *Die Pharmazie-An International Journal of Pharmaceutical Sciences*, *75*(2-3), 75-81.

[130] Ma, M.-Z., & Yu, Y. (2021). Research and Application Progress of Chitosan-based Hemostatic Materials---Review. *Zhongguo shi yan xue ye xue za zhi*, *29*(5), 1685-1689.

[131] Ma, X.-Y., Feng, Y.-F., Wang, T.-S., Lei, W., Li, X., Zhou, D.-P., Wen, X.-X., Yu, H.-L., Xiang, L.-B., & Wang, L. (2018). Involvement of FAK-mediated BMP-2/Smad pathway in mediating osteoblast adhesion and

differentiation on nano-HA/chitosan composite coated titanium implant under diabetic conditions. *Biomaterials Science*, *6*(1), 225-238.

[132] Ma, Y., Yao, J., Liu, Q., Han, T., Zhao, J., Ma, X., Tong, Y., Jin, G., Qu, K., Li, B., & Xu, F. (2020). Liquid Bandage Harvests Robust Adhesive, Hemostatic, and Antibacterial Performances as a First-Aid Tissue Adhesive. *Advanced Functional Materials*, *30*(39), 2001820. https://doi.org/https://doi.org/10.1002/adfm.202001820

[133] Magill, S. S., Edwards, J. R., Bamberg, W., Beldavs, Z. G., Dumyati, G., Kainer, M. A., Lynfield, R., Maloney, M., McAllister-Hollod, L., & Nadle, J. (2014). Multistate point-prevalence survey of health care–associated infections. *New England Journal of Medicine*, *370*(13), 1198-1208.

[134] Mandal, A., Clegg, J. R., Anselmo, A. C., & Mitragotri, S. (2020). Hydrogels in the clinic. *Bioengineering & Translational Medicine*, *5*(2), e10158. https://doi.org/https://doi.org/10.1002/btm2.10158

[135] Mao, H., Zhao, S., He, Y., Feng, M., Wu, L., He, Y., & Gu, Z. (2022). Multifunctional polysaccharide hydrogels for skin wound healing prepared by photoinitiator-free crosslinking. *Carbohydrate Polymers*, *285*, 119254.

[136] Martínez-Ruvalcaba, A., Chornet, E., & Rodrigue, D. (2007). Viscoelastic properties of dispersed chitosan/xanthan hydrogels. *Carbohydrate Polymers*, *67*(4), 586-595.

[137] Maschmeyer, T., Luque, R., & Selva, M. (2020). Upgrading of marine (fish and crustaceans) biowaste for high added-value molecules and bio (nano)-materials. *Chemical Society Reviews*, *49*(13), 4527-4563.

[138] Mattioli-Belmonte, M., Zizzi, A., Lucarini, G., Giantomassi, F., Biagini, G., Tucci, G., Orlando, F., Provinciali, M., Carezzi, F., & Morganti, P. (2007). Chitin nanofibrils linked to chitosan glycolate as spray, gel, and gauze preparations for wound repair. *Journal of bioactive and compatible polymers*, *22*(5), 525-538.

[139] Maturavongsadit, P., Paravyan, G., Shrivastava, R., & Benhabbour, S. R. (2020). Thermo-/pH-responsive chitosan-cellulose nanocrystals based hydrogel with tunable mechanical properties for tissue regeneration applications. *Materialia*, *12*, 100681. https://doi.org/https://doi.org/10.1016/j.mtla.2020.100681

[140] Merzendorfer, H. (2011). The cellular basis of chitin synthesis in fungi and insects: common principles and differences. *European journal of cell biology*, *90*(9), 759-769.

[141] Mezzana, P. (2008). Clinical efficacy of a new chitin nanofibrils-based gel in wound healing. *Acta chirurgiae plasticae*, *50*(3), 81-84.

[142] Miguel, S. P., Ribeiro, M. P., & Coutinho, P. (2021). Biomedical Applications of Biodegradable Polymers in Wound Care. In P. Kumar & V. Kothari (Eds.), *Wound Healing Research: Current Trends and Future Directions* (pp. 509-597). Springer Singapore. https://doi.org/10.1007/978-981-16-2677-7_17

[143] Mohite, P., Rahayu, P., Munde, S., Ade, N., Chidrawar, V. R., Singh, S., Jayeoye, T. J., Prajapati, B. G., Bhattacharya, S., & Patel, R. J. (2023). Chitosan-Based Hydrogel in the Management of Dermal Infections: A Review. *Gels*, *9*(7), 594.

[144] Mohraz, M., Golbabaei, F., Yu, I., Mansournia, M., Zadeh, A., & Dehghan, S. (2019). Preparation and optimization of multifunctional electrospun polyurethane/chitosan nanofibers for air pollution control applications. *International Journal of Environmental Science and Technology*, *16*, 681-694.

[145] Montaser, A., Rehan, M., & El-Naggar, M. E. (2019). pH-Thermosensitive hydrogel based on polyvinyl alcohol/sodium alginate/N-isopropyl acrylamide composite for treating re-infected wounds. *International Journal of Biological Macromolecules*, *124*, 1016-1024.

[146] Mousavi, S., Khoshfetrat, A. B., Khatami, N., Ahmadian, M., & Rahbarghazi, R. (2019). Comparative study of collagen and gelatin in chitosan-based hydrogels for effective wound dressing: Physical properties and fibroblastic cell behavior. *Biochemical and Biophysical Research Communications*, *518*(4), 625-631. https://doi.org/https://doi.org/10.1016/j.bbrc.2019.08.102

[147] Mozafari, M., Sefat, F., & Atala, A. (2019). *Handbook of tissue engineering scaffolds: Volume one*. Woodhead Publishing.

[148] Murakami, K., Aoki, H., Nakamura, S., Nakamura, S.-i., Takikawa, M., Hanzawa, M., Kishimoto, S., Hattori, H., Tanaka, Y., & Kiyosawa, T. (2010). Hydrogel blends of chitin/chitosan, fucoidan and alginate as healing-impaired wound dressings. *Biomaterials*, *31*(1), 83-90.

[149] Naderi Gharehgheshlagh, S., Fatemi, M. J., Jamili, S., Nourani, M. R., Sharifi, A. M., Saberi, M., Amini, N., & Ganji, F. (2021). A Dermal Gel Made of Rutilus Kutum Skin Collagen-Chitosan for Deep Burn Healing. *International Journal of Peptide Research and Therapeutics*, *27*(1), 317-328. https://doi.org/10.1007/s10989-020-10082-y

[150] Nikdel, M., Rajabinejad, H., Yaghoubi, H., Mikaeiliagah, E., Cella, M. A., Sadeghianmaryan, A., & Ahmadi, A. (2021). Fabrication of cellulosic nonwoven material coated with polyvinyl alcohol and zinc oxide/mesoporous silica nanoparticles for wound dressing purposes with cephalexin delivery. *ECS Journal of Solid State Science and Technology*, *10*(5), 057003.

[151] Ohmes, J., Saure, L. M., Schütt, F., Trenkel, M., Seekamp, A., Scherließ, R., Adelung, R., & Fuchs, S. (2022). Injectable Thermosensitive Chitosan-Collagen Hydrogel as A Delivery System for Marine Polysaccharide Fucoidan. *Marine Drugs*, *20*(6), 402. https://www.mdpi.com/1660-3397/20/6/402

[152] Oryan, A., Kamali, A., Moshiri, A., Baharvand, H., & Daemi, H. (2018). Chemical crosslinking of biopolymeric scaffolds: Current knowledge and future directions of crosslinked engineered bone scaffolds. *International Journal of Biological Macromolecules*, *107*, 678-688.

[153] Pan, H., Fan, D., Duan, Z., Zhu, C., Fu, R., & Li, X. (2019). Non-stick hemostasis hydrogels as dressings with bacterial barrier activity for cutaneous wound healing. *Materials Science and Engineering: C*, *105*, 110118. https://doi.org/https://doi.org/10.1016/j.msec.2019.110118

[154] Pang, L., Tian, P., Cui, X., Wu, X., Zhao, X., Wang, H., Wang, D., & Pan, H. (2021). In situ photo-cross-linking hydrogel accelerates diabetic wound healing through restored hypoxia-inducible factor 1-alpha pathway and regulated inflammation. *ACS Applied Materials & Interfaces*, *13*(25), 29363-29379.

[155] Peng, X., Liu, G., Zhu, L., Yu, K., Qian, K., & Zhan, X. (2020). In vitro and in vivo study of novel antimicrobial gellan–polylysine polyion complex fibers as suture materials. *Carbohydrate Research*, *496*, 108115. https://doi.org/https://doi.org/10.1016/j.carres.2020.108115

[156] Pietraszek, A., Ledwójcik, G., Lewandowska-Łańcucka, J., Horak, W., Lach, R., Łatkiewicz, A., & Karewicz, A. (2020). Bioactive hydrogel scaffolds reinforced with alkaline-phosphatase containing halloysite nanotubes for bone repair applications. *International Journal of Biological Macromolecules*, *163*, 1187-1195. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2020.07.045

[157] Putra, A. P., Fahmadiyah, E., Hikmawati, D., & Aminatun, A. (2020). Chitosan-Coated Collagen-Hyaluronic Acid-Poly Ethylene Oxide-Based Electrospun Membrane for Corneal Ulcers Wound Dressing Candidate. *Walailak Journal of Science and Technology (WJST)*, *17*(11), 1230-1240. https://doi.org/10.48048/wjst.2021.6319

[158] Qasim, M., Udomluck, N., Chang, J., Park, H., & Kim, K. (2018). RETRACTED ARTICLE: Antimicrobial activity of silver nanoparticles encapsulated in poly-N-isopropylacrylamide-based polymeric nanoparticles. *International journal of nanomedicine*, 235-249.

[159] Qian, C., Higashigaki, T., Asoh, T.-A., & Uyama, H. (2020). Anisotropic conductive hydrogels with high water content. *ACS Applied Materials & Interfaces*, *12*(24), 27518-27525.

[160] Qianqian, O., Songzhi, K., Yongmei, H., Xianghong, J., Sidong, L., Puwang, L., & Hui, L. (2021). Preparation of nano-hydroxyapatite/chitosan/tilapia skin peptides hydrogels and its burn wound treatment. *International Journal of Biological Macromolecules*, *181*, 369-377. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2021.03.085

[161] Qin, H., & Wang, K. (2019). Study on preparation and performance of PEG-based polyurethane foams modified by the chitosan with different molecular weight. *International Journal of Biological Macromolecules*, *140*, 877-885.

[162] Qing, X., He, G., Liu, Z., Yin, Y., Cai, W., Fan, L., & Fardim, P. (2021). Preparation and properties of polyvinyl alcohol/N–succinyl chitosan/lincomycin composite antibacterial hydrogels for wound dressing. *Carbohydrate Polymers*, *261*, 117875.

[163] Qun, G., & Ajun, W. (2006). Effects of molecular weight, degree of acetylation and ionic strength on surface tension of chitosan in dilute solution. *Carbohydrate Polymers*, *64*(1), 29-36.

[164] Radulescu, D.-M., Neacsu, I. A., Grumezescu, A.-M., & Andronescu, E. (2022). New insights of scaffolds based on hydrogels in tissue engineering. *Polymers*, *14*(4), 799.

[165] Raj, R. M., Priya, P., & Raj, V. (2018). Gentamicin-loaded ceramic-biopolymer dual layer coatings on the Ti with improved bioactive and corrosion resistance properties for orthopedic applications. *Journal of the mechanical behavior of biomedical materials*, *82*, 299-309.

[166] Rana, M. M., Rahman, M. S., Ullah, M. A., Siddika, A., Hossain, M. L., Akhter, M. S., Hasan, M. Z., & Asaduzzaman, S. M. (2020). Amnion and collagen-based blended hydrogel improves burn healing efficacy on a rat skin wound model in the presence of wound dressing biomembrane. *Bio-Medical Materials and Engineering*, *31*(1), 1-17.

[167] Raus, R. A., Nawawi, W. M. F. W., & Nasaruddin, R. R. (2021). Alginate and alginate composites for biomedical applications. *Asian Journal of Pharmaceutical Sciences*, *16*(3), 280-306.

[168] Rawat, P., Zhu, D., Rahman, M. Z., & Barthelat, F. (2021). Structural and mechanical properties of fish scales for the bio-inspired design of flexible body armors: A review. *Acta biomaterialia*, *121*, 41-67.

[169] Reyna-Urrutia, V. A., Mata-Haro, V., Cauich-Rodriguez, J. V., Herrera-Kao, W. A., & Cervantes-Uc, J. M. (2019). Effect of two crosslinking methods on the physicochemical and biological properties of the collagen-chitosan scaffolds. *European Polymer Journal*, *117*, 424-433. https://doi.org/https://doi.org/10.1016/j.eurpolymj.2019.05.010

[170] Rezaei, A., Aligholi, H., Zeraatpisheh, Z., Gholami, A., & Mirzaei, E. (2021). Collagen/chitosan-functionalized graphene oxide hydrogel provide a 3D matrix for neural stem/precursor cells survival, adhesion, infiltration and migration. *Journal of bioactive and compatible polymers*, *36*(4), 296-313.

[171] Ribeiro, D. M. L., Carvalho Júnior, A. R., Vale de Macedo, G. H. R., Chagas, V. L., Silva, L. d. S., Cutrim, B. d. S., Santos, D. M., Soares, B. L. L., Zagmignan, A., de Miranda, R. d. C. M., de Albuquerque, P. B. S., & Nascimento da Silva, L. C. (2020). Polysaccharide-Based Formulations for Healing of Skin-Related Wound Infections: Lessons from Animal Models and Clinical Trials. *Biomolecules*, *10*(1), 63. https://www.mdpi.com/2218-273X/10/1/63

[172] Rizeq, B. R., Younes, N. N., Rasool, K., & Nasrallah, G. K. (2019). Synthesis, bioapplications, and toxicity evaluation of chitosan-based nanoparticles. *International Journal of Molecular Sciences*, *20*(22), 5776.

[173] Salati, M. A., Khazai, J., Tahmuri, A. M., Samadi, A., Taghizadeh, A., Taghizadeh, M., Zarrintaj, P., Ramsey, J. D., Habibzadeh, S., & Seidi, F. (2020). Agarose-based biomaterials: Opportunities and challenges in cartilage tissue engineering. *Polymers*, *12*(5), 1150.

[174] Sánchez-Cid, P., Jiménez-Rosado, M., Rubio-Valle, J. F., Romero, A., Ostos, F. J., Rafii-El-Idrissi Benhnia, M., & Perez-Puyana, V. (2022). Biocompatible and Thermoresistant Hydrogels Based on Collagen and Chitosan. *Polymers*, *14*(2), 272. https://www.mdpi.com/2073-4360/14/2/272

[175] Sareethammanuwat, M., Boonyuen, S., & Arpornmaeklong, P. (2021). Effects of beta-tricalcium phosphate nanoparticles on the properties of a thermosensitive chitosan/collagen hydrogel and controlled release of

quercetin. *Journal of Biomedical Materials Research Part A*, *109*(7), 1147-1159. https://doi.org/https://doi.org/10.1002/jbm.a.37107

[176] Schagen, S. K. (2017). Topical peptide treatments with effective anti-aging results. *Cosmetics*, *4*(2), 16.

[177] Sezlev Bilecen, D., Uludag, H., & Hasirci, V. (2019). Development of PEI-RANK siRNA complex loaded PLGA nanocapsules for the treatment of osteoporosis. *Tissue Engineering Part A*, *25*(1-2), 34-43.

[178] Shagdarova, B., Konovalova, M., Zhuikova, Y., Lunkov, A., Zhuikov, V., Khaydapova, D., Il'ina, A., Svirshchevskaya, E., & Varlamov, V. (2022). Collagen/Chitosan Gels Cross-Linked with Genipin for Wound Healing in Mice with Induced Diabetes. *Materials*, *15*(1), 15. https://www.mdpi.com/1996-1944/15/1/15

[179] Shah, R., Stodulka, P., Skopalova, K., & Saha, P. (2019). Dual Crosslinked Collagen/Chitosan Film for Potential Biomedical Applications. *Polymers*, *11*(12), 2094. https://www.mdpi.com/2073-4360/11/12/2094

[180] Sharma, P. K., Halder, M., Srivastava, U., & Singh, Y. (2019). Antibacterial PEG-chitosan hydrogels for controlled antibiotic/protein delivery. *ACS applied bio materials*, *2*(12), 5313-5322.

[181] Sheng, C., Zhou, Y., Lu, J., Zhang, X., & Xue, G. (2019). Preparation and characterization of chitosan based hydrogels chemical cross-linked by oxidized cellulose nanowhiskers. *Polymer Composites*, *40*(6), 2432-2440.

[182] Shi, Q., Rondon-Cavanzo, E.-P., Dalla Picola, I. P., Tiera, M. J., Zhang, X., Dai, K., Benabdoune, H. A., Benderdour, M., & Fernandes, J. C. (2018). In vivo therapeutic efficacy of TNFα silencing by folate-PEG-chitosan-DEAE/siRNA nanoparticles in arthritic mice. *International journal of nanomedicine*, 387-402.

[183] Shin, J.-W., Kwon, S.-H., Choi, J.-Y., Na, J.-I., Huh, C.-H., Choi, H.-R., & Park, K.-C. (2019). Molecular mechanisms of dermal aging and antiaging approaches. *International Journal of Molecular Sciences*, *20*(9), 2126.

[184] Si, J., Yang, Y., Xing, X., Yang, F., & Shan, P. (2019). Controlled degradable chitosan/collagen composite scaffolds for application in nerve tissue regeneration. *Polymer Degradation and Stability*, *166*, 73-85. https://doi.org/https://doi.org/10.1016/j.polymdegradstab.2019.05.023

[185] Silvipriya, K., Kumar, K. K., Bhat, A., Kumar, B. D., & John, A. (2015). Collagen: Animal sources and biomedical application. *Journal of Applied Pharmaceutical Science*, *5*(3), 123-127.

[186] Singh, Y. P., Bhardwaj, N., & Mandal, B. B. (2016). Potential of agarose/silk fibroin blended hydrogel for in vitro cartilage tissue engineering. *ACS Applied Materials & Interfaces*, *8*(33), 21236-21249.

[187] Sionkowska, A., Lewandowska, K., & Adamiak, K. (2020). The influence of UV light on rheological properties of collagen extracted from Silver Carp skin. *Materials*, *13*(19), 4453.

[188] Sklenářová, R., Akla, N., Latorre, M. J., Ulrichová, J., & Franková, J. (2022). Collagen as a biomaterial for skin and corneal wound healing. *Journal of Functional Biomaterials*, *13*(4), 249.

[189] Smith, I. P., Domingos, M., Richardson, S. M., & Bella, J. (2023). Characterization of the Biophysical Properties and Cell Adhesion Interactions of Marine Invertebrate Collagen from Rhizostoma pulmo. *Marine Drugs*, *21*(2), 59.

[190] Socrates, R., Prymak, O., Loza, K., Sakthivel, N., Rajaram, A., Epple, M., & Narayana Kalkura, S. (2019). Biomimetic fabrication of mineralized composite films of nanosilver loaded native fibrillar collagen and chitosan. *Materials Science and Engineering: C*, *99*, 357-366. https://doi.org/https://doi.org/10.1016/j.msec.2019.01.101

[191] Song, K., Qiao, M., Liu, T., Jiang, B., Macedo, H. M., Ma, X., & Cui, Z. (2010). Preparation, fabrication and biocompatibility of novel injectable temperature-sensitive chitosan/glycerophosphate/collagen hydrogels. *Journal of Materials Science: Materials in Medicine*, *21*(10), 2835-2842. https://doi.org/10.1007/s10856-010-4131-4

[192] Song, W.-Y., Liu, G.-M., Li, J., & Luo, Y.-G. (2016). Bone morphogenetic protein-2 sustained delivery by hydrogels with microspheres repairs rabbit mandibular defects. *Tissue engineering and regenerative medicine*, *13*, 750-761.

[193] Sparks, H. D., Sigaeva, T., Tarraf, S., Mandla, S., Pope, H., Hee, O., Di Martino, E. S., Biernaskie, J., Radisic, M., & Scott, W. M. (2021). Biomechanics of Wound Healing in an Equine Limb Model: Effect of Location and Treatment with a Peptide-Modified Collagen–Chitosan Hydrogel. *ACS Biomaterials Science & Engineering*, *7*(1), 265-278. https://doi.org/10.1021/acsbiomaterials.0c01431

[194] Stepanovska, J., Otahal, M., Hanzalek, K., Supova, M., & Matejka, R. (2021). pH modification of high-concentrated collagen bioinks as a factor affecting cell viability, mechanical properties, and printability. *Gels*, *7*(4), 252.

[195] Su, J., Li, J., Liang, J., Zhang, K., & Li, J. (2021). Hydrogel preparation methods and biomaterials for wound dressing. *Life*, *11*(10), 1016.

[196] Subhan, F., Ikram, M., Shehzad, A., & Ghafoor, A. (2015). Marine collagen: An emerging player in biomedical applications. *Journal of food science and technology*, *52*, 4703-4707.

[197] Sudheesh Kumar, P., Lakshmanan, V.-K., Anilkumar, T., Ramya, C., Reshmi, P., Unnikrishnan, A., Nair, S. V., & Jayakumar, R. (2012). Flexible and microporous chitosan hydrogel/nano ZnO composite bandages for wound dressing: in vitro and in vivo evaluation. *ACS Applied Materials & Interfaces*, *4*(5), 2618-2629.

[198] Sun, H., Zhang, M., Liu, M., Yu, Y., Xu, X., & Li, J. (2020). Fabrication of double-network hydrogels with universal adhesion and superior extensibility and cytocompatibility by one-pot method. *Biomacromolecules*, *21*(12), 4699-4708.

[199] Takeya, H., Itai, S., Kimura, H., Kurashina, Y., Amemiya, T., Nagoshi, N., Iwamoto, T., Sato, K., Shibata, S., Matsumoto, M., Onoe, H., & Nakamura, M. (2023). Schwann cell-encapsulated chitosan-collagen hydrogel nerve conduit promotes peripheral nerve regeneration in rodent sciatic nerve defect models. *Scientific Reports*, *13*(1), 11932. https://doi.org/10.1038/s41598-023-39141-2

[200] Tan, L., Hu, Y., Li, M., Zhang, Y., Xue, C., Chen, M., Luo, Z., & Cai, K. (2022). Remotely-activatable extracellular matrix-mimetic hydrogel promotes physiological bone mineralization for enhanced cranial defect healing. *Chemical Engineering Journal*, *431*, 133382. https://doi.org/https://doi.org/10.1016/j.cej.2021.133382

[201] Teotia, R. S., Kalita, D., Singh, A. K., Verma, S. K., Kadam, S. S., & Bellare, J. R. (2015). Bifunctional polysulfone-chitosan composite hollow fiber membrane for bioartificial liver. *ACS Biomaterials Science & Engineering*, *1*(6), 372-381.

[202] Thomas, V., Yallapu, M. M., Sreedhar, B., & Bajpai, S. (2007). A versatile strategy to fabricate hydrogel–silver nanocomposites and investigation of their antimicrobial activity. *Journal of colloid and interface science*, *315*(1), 389-395.

[203] Thongchai, K., Chuysinuan, P., Thanyacharoen, T., Techasakul, S., & Ummartyotin, S. (2020). Characterization, release, and antioxidant activity of caffeic acid-loaded collagen and chitosan hydrogel composites. *Journal of Materials Research and Technology*, *9*(3), 6512-6520. https://doi.org/https://doi.org/10.1016/j.jmrt.2020.04.036

[204] Tiplea, R. E., Lemnaru, G.-M., Trusca, R., Holban, A., Kaya, M. G. A., Dragu, L. D., Ficai, D., Ficai, A., & Bleotu, C. (2021). Antimicrobial films based on chitosan, collagen, and zno for skin tissue regeneration. *Biointerface Res. Appl. Chem*, *11*, 11985-11995.

[205] Toshniwal, P., Nguyen, M., Guédin, A., Viola, H., Ho, D., Kim, Y., Bhatt, U., Bond, C. S., Hool, L., & Hurley, L. H. (2019). TGF-β-induced fibrotic stress increases G-quadruplex formation in human fibroblasts. *FEBS letters*, *593*(22), 3149-3161.

[206] Tran, N. Q., Joung, Y. K., Lih, E., & Park, K. D. (2011). In situ forming and rutin-releasing chitosan hydrogels as injectable dressings for dermal wound healing. *Biomacromolecules*, *12*(8), 2872-2880.

[207] Tripathy, A., Pahal, S., Mudakavi, R. J., Raichur, A. M., Varma, M. M., & Sen, P. (2018). Impact of Bioinspired Nanotopography on the Antibacterial and Antibiofilm Efficacy of Chitosan. *Biomacromolecules*, *19*(4), 1340-1346. https://doi.org/10.1021/acs.biomac.8b00200

[208] Trombino, S., Servidio, C., Curcio, F., & Cassano, R. (2019). Strategies for hyaluronic acid-based hydrogel design in drug delivery. *Pharmaceutics*, *11*(8), 407.

[209] Umar, A. K., Luckanagul, J. A., Zothantluanga, J. H., & Sriwidodo, S. (2022). Complexed Polymer Film-Forming Spray: An Optimal Delivery System for Secretome of Mesenchymal Stem Cell as Diabetic Wound Dressing? *Pharmaceuticals*, *15*(7), 867. https://www.mdpi.com/1424-8247/15/7/867

[210] Utech, S., & Boccaccini, A. R. (2016). A review of hydrogel-based composites for biomedical applications: enhancement of hydrogel properties by addition of rigid inorganic fillers. *Journal of Materials Science*, *51*, 271-310.

[211] Valentino, C., Vigani, B., Zucca, G., Ruggeri, M., Boselli, C., Icaro Cornaglia, A., Malavasi, L., Sandri, G., & Rossi, S. (2023). Formulation development of collagen/chitosan-based porous scaffolds for skin wounds repair and regeneration. *International Journal of Biological Macromolecules*, *242*, 125000. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2023.125000

[212] Valipour, F., Rahimabadi, E. Z., & Rostamzad, H. (2023). Preparation and characterization of wound healing hydrogel based on fish skin collagen and chitosan cross-linked by dialdehyde starch. *International Journal of Biological Macromolecules*, *253*, 126704. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2023.126704

[213] Vaneau, M., Chaby, G., Guillot, B., Martel, P., Senet, P., Téot, L., & Chosidow, O. (2007). Consensus panel recommendations for chronic and acute wound dressings. *Archives of dermatology*, *143*(10), 1291-1294.

[214] Varghese, S. A., Rangappa, S. M., Siengchin, S., & Parameswaranpillai, J. (2020). Natural polymers and the hydrogels prepared from them. In *Hydrogels based on natural polymers* (pp. 17-47). Elsevier.

[215] Varoni, E., Tschon, M., Palazzo, B., Nitti, P., Martini, L., & Rimondini, L. (2012). Agarose gel as biomaterial or scaffold for implantation surgery: characterization, histological and histomorphometric study on soft tissue response. *Connective tissue research*, *53*(6), 548-554.

[216] Vázquez, J. A., Rodríguez-Amado, I., Montemayor, M. I., Fraguas, J., del Pilar González, M., & Murado, M. A. (2013). Chondroitin sulfate, hyaluronic acid and chitin/chitosan production using marine waste sources: Characteristics, applications and eco-friendly processes: A review. *Marine Drugs*, *11*(3), 747-774.

[217] Veeruraj, A., Arumugam, M., & Balasubramanian, T. (2013). Isolation and characterization of thermostable collagen from the marine eel-fish (Evenchelys macrura). *Process biochemistry*, *48*(10), 1592-1602.

[218] Wang, B., Wang, Y.-M., Chi, C.-F., Luo, H.-Y., Deng, S.-G., & Ma, J.-Y. (2013). Isolation and characterization of collagen and antioxidant collagen peptides from scales of croceine croaker (Pseudosciaena crocea). *Marine Drugs*, *11*(11), 4641-4661.

[219] Wang, D., Yang, H., Zhou, Z., Zhao, M., Chen, R., & Reed, S. H. (2018). XPF plays an indispensable role in relieving silver nanoparticle induced DNA damage stress in human cells. *Toxicology Letters*, *288*, 44-54.

[220] Wang, D., Zhang, N., Meng, G., He, J., & Wu, F. (2020). The effect of form of carboxymethyl-chitosan dressings on biological properties in wound healing. *Colloids and Surfaces B: Biointerfaces*, *194*, 111191. https://doi.org/https://doi.org/10.1016/j.colsurfb.2020.111191

[221] Wang, L., An, X., Yang, F., Xin, Z., Zhao, L., & Hu, Q. (2008). Isolation and characterisation of collagens from the skin, scale and bone of deep-sea redfish (Sebastes mentella). *Food chemistry*, *108*(2), 616-623.

[222] Wang, L., Liang, Q., Chen, T., Wang, Z., Xu, J., & Ma, H. (2014). Characterization of collagen from the skin of Amur sturgeon (Acipenser schrenckii). *Food Hydrocolloids*, *38*, 104-109.

[223] Wang, L., & Stegemann, J. P. (2010). Thermogelling chitosan and collagen composite hydrogels initiated with β-glycerophosphate for bone tissue engineering. *Biomaterials*, *31*(14), 3976-3985. https://doi.org/https://doi.org/10.1016/j.biomaterials.2010.01.131

[224] Wang, L., & Stegemann, J. P. (2011). Glyoxal crosslinking of cell-seeded chitosan/collagen hydrogels for bone regeneration. *Acta biomaterialia*, *7*(6), 2410-2417.

[225] Wang, Y., Zhang, Y., Su, J., Zhang, X., Wang, J., & Tu, Q. (2020). Preparation of a multifunctional wound dressing based on a natural deep eutectic solvent. *ACS Sustainable Chemistry & Engineering*, *8*(37), 14243-14252.

[226] Wang, Z., Nie, J., Qin, W., Hu, Q., & Tang, B. Z. (2016). Gelation process visualized by aggregation-induced emission fluorogens. *Nature communications*, *7*(1), 12033.

[227] Wei, L., Tan, J., Li, L., Wang, H., Liu, S., Chen, J., Weng, Y., & Liu, T. (2022). Chitosan/alginate hydrogel dressing loaded FGF/VE-cadherin to accelerate full-thickness skin regeneration and more normal skin repairs. *International Journal of Molecular Sciences*, *23*(3), 1249.

[228] Wei, M., Gao, Y., Li, X., & Serpe, M. (2017). Stimuli-Responsive Polymers and Their Applications. Polym. Chem. 2017, 8 (1), 127–143. In.

[229] Woraphatphadung, T., Sajomsang, W., Rojanarata, T., Ngawhirunpat, T., Tonglairoum, P., & Opanasopit, P. (2018). Development of chitosan-based pH-sensitive polymeric micelles containing curcumin for colon-targeted drug delivery. *AAPS PharmSciTech*, *19*, 991-1000.

[230] Wu, Q., Maire, M., Lerouge, S., Therriault, D., & Heuzey, M. C. (2017). 3D printing of microstructured and stretchable chitosan hydrogel for guided cell growth. *Advanced Biosystems*, *1*(6), 1700058.

[231] Xie, H., Chen, X., Shen, X., He, Y., Chen, W., Luo, Q., Ge, W., Yuan, W., Tang, X., Hou, D., Jiang, D., Wang, Q., Liu, Y., Liu, Q., & Li, K. (2018). Preparation of chitosan-collagen-alginate composite dressing and its promoting effects on wound healing. *International Journal of Biological Macromolecules*, *107*, 93-104. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2017.08.142

[232] Xu, L., Liu, Y., Tang, L., Xiao, H., Yang, Z., & Wang, S. (2022). Preparation of recombinant human collagen III protein hydrogels with sustained release of extracellular vesicles for skin wound healing. *International Journal of Molecular Sciences*, *23*(11), 6289.

[233] Xu, Y., Chen, H., Fang, Y., & Wu, J. (2022). Hydrogel Combined with Phototherapy in Wound Healing. *Advanced Healthcare Materials*, *11*(16), 2200494. https://doi.org/https://doi.org/10.1002/adhm.202200494

[234] Yamada, Y., Yoshida, C., Hamada, K., Kikkawa, Y., & Nomizu, M. (2020). Development of three-dimensional cell culture scaffolds using laminin peptide-conjugated agarose microgels. *Biomacromolecules*, *21*(9), 3765-3771.

[235] Yang, C., Dan, N., You, W., Huang, Y., Chen, Y., Yu, G., Dan, W., & Wen, H. (2019). Modification of collagen-chitosan membrane by oxidation sodium alginate and in vivo/ in vitro evaluation for wound dressing application. *International Journal of Polymer Analysis and Characterization*, *24*(7), 619-629. https://doi.org/10.1080/1023666X.2019.1648637

[236] Yang, M., He, S., Su, Z., Yang, Z., Liang, X., & Wu, Y. (2020). Thermosensitive Injectable Chitosan/Collagen/β-Glycerophosphate Composite Hydrogels for Enhancing Wound Healing by Encapsulating Mesenchymal Stem Cell Spheroids. *ACS Omega*, *5*(33), 21015-21023. https://doi.org/10.1021/acsomega.0c02580

[237] Yang, Y., Xu, R., Wang, C., Guo, Y., Sun, W., & Ouyang, L. (2022). Recombinant human collagen-based bioinks for the 3D bioprinting of full-thickness human skin equivalent. *International Journal of Bioprinting*, *8*(4).

[238] Yi, Y., Wang, Y., & Liu, H. (2003). Preparation of new crosslinked chitosan with crown ether and their adsorption for silver ion for antibacterial activities. *Carbohydrate Polymers*, *53*(4), 425-430.

[239] Ying, H., Zhou, J., Wang, M., Su, D., Ma, Q., Lv, G., & Chen, J. (2019). In situ formed collagen-hyaluronic acid hydrogel as biomimetic dressing for promoting spontaneous wound healing. *Materials Science and Engineering: C*, *101*, 487-498.

[240] Zavyalova, O., Gajewska, S., Dąbrowska-Wisłocka, D., & Sionkowska, A. (2021). Characteristics of physicochemical and rheological properties of chitosan hydrogels based on selected hydroxy acids. *Engineering of Biomaterials*, *24*(161).

[241] Zhang, J., Xie, R., Zhang, S.-B., Cheng, C.-J., Ju, X.-J., & Chu, L.-Y. (2009). Rapid pH/temperature-responsive cationic hydrogels with dual stimuli-sensitive grafted side chains. *Polymer*, *50*(11), 2516-2525.

[242] Zhang, K., Chen, X., Xue, Y., Lin, J., Liang, X., Zhang, J., Zhang, J., Chen, G., Cai, C., & Liu, J. (2022). Tough Hydrogel Bioadhesives for Sutureless Wound Sealing, Hemostasis and Biointerfaces. *Advanced Functional Materials*, *32*(15), 2111465. https://doi.org/https://doi.org/10.1002/adfm.202111465

[243] Zhang, M.-X., Zhao, W.-Y., Fang, Q.-Q., Wang, X.-F., Chen, C.-Y., Shi, B.-H., Zheng, B., Wang, S.-J., Tan, W.-Q., & Wu, L.-H. (2021). Effects of chitosan-collagen dressing on wound healing in vitro and in vivo assays. *Journal of Applied Biomaterials & Functional Materials*, *19*, 2280800021989698. https://doi.org/10.1177/2280800021989698

[244] Zhao, H., Huang, J., Li, Y., Lv, X., Zhou, H., Wang, H., Xu, Y., Wang, C., Wang, J., & Liu, Z. (2020). ROS-scavenging hydrogel to promote healing of bacteria infected diabetic wounds. *Biomaterials*, *258*, 120286.

[245] Zhao, L., Li, X., Zhao, J., Ma, S., Ma, X., Fan, D., Zhu, C., & Liu, Y. (2016). A novel smart injectable hydrogel prepared by microbial transglutaminase and human-like collagen: Its characterization and biocompatibility. *Materials Science and Engineering: C*, *68*, 317-326.

[246] Zhao, N., & Yuan, W. (2022). Functionally integrated bioglass microspheres-composited double-network hydrogel with good tissue adhesion and electrical conductivity for efficient wound treatment and health detection. *Composites Part B: Engineering*, *242*, 110095.

[247] Zhao, X., Zhou, L., Riaz Rajoka, M. S., Yan, L., Jiang, C., Shao, D., Zhu, J., Shi, J., Huang, Q., & Yang, H. (2018). Fungal silver nanoparticles: synthesis, application and challenges. *Critical reviews in biotechnology*, *38*(6), 817-835.

[248] Zheng, Z., Qi, J., Hu, L., Ouyang, D., Wang, H., Sun, Q., Lin, L., You, L., & Tang, B. (2022). A cannabidiol-containing alginate based hydrogel as novel multifunctional wound dressing for promoting wound healing. *Biomaterials Advances*, *134*, 112560.

[249] Zhong, Y., Xiao, H., Seidi, F., & Jin, Y. (2020). Natural polymer-based antimicrobial hydrogels without synthetic antibiotics as wound dressings. *Biomacromolecules*, *21*(8), 2983-3006.

[250] Zhou, P., Li, X., Zhang, B., Shi, Q., Li, D., & Ju, X. (2019). A human umbilical cord mesenchymal stem cell-conditioned medium/chitosan/collagen/β-glycerophosphate thermosensitive hydrogel promotes burn injury healing in mice. *BioMed research international*, *2019*.

[251] Zhu, C., Lei, H., Fan, D., Duan, Z., Li, X., Li, Y., Cao, J., Wang, S., & Yu, Y. (2018). Novel enzymatic crosslinked hydrogels that mimic extracellular matrix for skin wound healing. *Journal of Materials Science*, *53*(8), 5909-5928. https://doi.org/10.1007/s10853-017-1956-y

[252] Zhu, J., Han, H., Li, F., Wang, X., Yu, J., Chu, C.-C., & Wu, D. (2019). Self-assembly of amino acid-based random copolymers for antibacterial application and infection treatment as nanocarriers. *Journal of colloid and interface science*, *540*, 634-646.

[253] Zhu, J., Jiang, G., Song, G., Liu, T., Cao, C., Yang, Y., Zhang, Y., & Hong, W. (2019). Incorporation of ZnO/Bioactive Glass Nanoparticles into Alginate/Chitosan Composite Hydrogels for Wound Closure. *ACS Applied Bio Materials*, *2*(11), 5042-5052. https://doi.org/10.1021/acsabm.9b00727

[254] Zubairi, W., Tehseen, S., Nasir, M., Anwar Chaudhry, A., Ur Rehman, I., & Yar, M. (2022). A study of the comparative effect of cerium oxide and cerium peroxide on stimulation of angiogenesis: Design and synthesis of pro-angiogenic chitosan/collagen hydrogels. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *110*(12), 2751-2762. https://doi.org/https://doi.org/10.1002/jbm.b.35126