

Development of a Biocompatible and Biodegradable Chitosan-Gelatin Scaffold Crosslinked to
Enhance Wound Healing and Antimicrobial Activity

By

Benjamin Louis Wilczewski

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John S Wilbur

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Abstract

Traditional bandages and gauze are widely used in medical applications but suffer from significant limitations, including poor biocompatibility and the absence of properties that would aid in wound healing. This study proposes the development of a chitosan-gelatin scaffold crosslinked with genipin to address these shortcomings. The resulting scaffold is designed to be biodegradable and biocompatible while offering antibacterial, hemostatic, anti-inflammatory, and antifungal properties, all of which are critical for enhancing wound healing and promoting tissue regeneration. Although no experimental data have been collected, this proposal is based on an extensive review of studies involving chitosan, gelatin, crosslinking agents, and related scaffolds, culminating in a detailed procedural framework for future research and development.

Introduction

Traumatic injuries often occur unexpectedly, presenting significant challenges in terms of immediate treatment, particularly in settings where access to medical facilities is limited. In rural communities, for instance, where hospitals may not be easily accessible, the management of traumatic injuries becomes even more critical. While products such as traditional gauze, antiseptics, wound dressings, and ointments are commonly used, there remains a clear need for more effective and protective solutions that can be readily deployed in these circumstances. One promising approach is the development of a novel gauze composed of chitosan and gelatin.

Chitosan is a naturally occurring polysaccharide derived from chitin, which is found in the exoskeletons of various animals, insects, and the cell walls of certain fungi (Rajinikanth B).

Gelatin, a protein obtained from collagen, is rich in amino acids and possesses several properties

that make it particularly suitable for wound healing. Both chitosan and gelatin are FDA-approved and biodegradable, offering the advantage of being safely applied to wounds where they can remain in place while providing antibacterial effects and promoting clot formation (Deng, Chun-Mei).

The production of gauze made from these materials offers an innovative and cost-effective solution to improve wound care, especially in resource-limited settings. Such a product would not only reduce the risk of infection but also minimize tissue and organ damage. In areas where medical assistance may be scarce—such as rural regions, remote outdoor environments, and combat zones—the risk of infection and severe injury is significantly heightened. Therefore, the development of a portable, easily stored, and efficient wound care product is crucial to improving outcomes and reducing mortality and organ loss in these high-risk populations. Additionally, this chitosan-gelatin gauze could have significant applications in the surgical and medical fields, where its biocompatibility, antimicrobial properties, and ability to promote hemostasis make it an ideal candidate for use in wound closure, tissue regeneration, and as a surgical dressing for post-operative care (Deng, Chun-Mei).

Chitosan

Chitosan, derived through the deacetylation of chitin, has emerged as a promising biomaterial for tissue regeneration and wound healing due to its desirable properties, including biocompatibility, biodegradability, affinity for biomolecules, and its ability to promote wound healing (Rajinikanth B). As a natural polymer, chitosan facilitates the attraction of new cells and engages

synergistically with the body, promoting accelerated healing, tissue and skin regeneration, as well as reducing inflammation and infection (Rajinikanth B).

A primary concern with many current medical scaffolds and loading devices is their lack of biocompatibility. These non-compatible devices often adhere to lesions or wounds, necessitating mechanical removal, which can disrupt wound healing by damaging newly formed tissues (Lungu). In contrast, chitosan is not only biocompatible but also biodegradable, allowing medical devices made from this material to degrade gradually within the host without producing toxic byproducts. As a hydrophilic polymer, chitosan degrades through enzymatic hydrolysis by lysozymes, bacterial enzymes, and three active chitinases, resulting in the formation of amino sugars that can be processed and excreted by the metabolic system (Rodriguez, Ul-Islam). The degradation rate of chitosan can be modulated by its degree of deacetylation, with a higher degree of deacetylation reducing the degradation rate (Kean, Rodriguez). Although chitosan is biodegradable, it is a weak base and insoluble in water and organic solvents, however, it dissolves in acidic environments ($pK_a < 6.5$) (Gheorghiuță). To overcome this limitation, chitosan is often combined with more soluble materials, such as gelatin, to enhance its solubility.

Chitosan is also considered non-toxic and has been approved for dietary use in several countries, including Japan, Italy, and Finland, as well as being FDA-approved for use in wound dressings (Kean). Multiple *in vitro* studies have shown that chitosan, at varying molecular weights, exhibits minimal cytotoxicity against human lymphoblastic leukemia and human embryonic lung cells (Kean). Moreover, an *in vitro* study on red cell hemolysis at different chitosan molecular weights found no evidence of red blood cell lysis (Richardson S, Zhang). While a study by Carreño-Gomez et al. suggested relative toxicity, this was likely due to the inappropriate pH conditions used in the assay, which compromised cell viability (Carreño-Gomez, Kean).

Conversely, in vivo studies have consistently shown no toxic effects following the implantation of chitosan-based devices (Kean). In a study using 200 μL of 30 mg/mL photo-crosslinked azide–chitosan–lactose gel, it was found that the chitosan-based implants not only exhibited no toxicity, but also enhanced wound contraction and healing (Azad). Overall, chitosan has been demonstrated to be non-toxic and biocompatible in its pure form, though caution is warranted when incorporating additional components, such as metal oxides or coatings into the material (Kean).

The chemical structure of chitosan further contributes to its suitability as a biomaterial. Chitosan consists of a polysaccharide backbone with β -(1 \rightarrow 4)-linked D-glucosamine and N-acetyl-D-glucosamine units. These sugar units alternate in a 180° rotation, forming the disaccharide N, N'-diacetylchitobiose (Elieh-Ali-Komi). The polymer chains adopt a helical structure due to the inversion of neighboring sugar units, which enhances stability, flexibility, and strength through hydrogen bonding (Elieh-Ali-Komi). The presence of crystalline allomorphs (α -, β -, and γ -) further improves the material's stability and flexibility. The most common form, α -chitin, features antiparallel polymer chains held together by hydrogen bonds, which promote intra- and intermolecular bonding, thereby enhancing stability (Elieh-Ali-Komi). β -chitin, although less common, has parallel poly-N-acetylglucosamine chains and lacks inter-sheet hydrogen bonds, which provides greater flexibility. γ -chitin exhibits a mix of parallel and antiparallel orientations (Elieh-Ali-Komi). The crystallinity of chitosan offers a balance between rigidity and flexibility, which can be fine-tuned by adjusting its degree of acetylation (Elieh-Ali-Komi). Despite being generally less flexible and more rigid compared to other biomaterials, chitosan's modifiability enables chemical adjustments—such as adding crosslinking agents to enhance elasticity and resistance, or altering pore size—to optimize its physical properties for specific applications

(Rodriguez, Elieh-Ali-Komi, Józwiak). These modifications can affect degradation time, structural stability, nutrient diffusion, cell differentiation, and mechanical strength (Elieh-Ali-Komi, Józwiak). Chitosan's structure provides excellent biomechanical properties for scaffolds, supporting growth factor delivery and nutrient and oxygen diffusion (Ikeda). Additionally, its hydrophilic nature promotes cell adhesion, making it ideal for tissue engineering (Rodriguez).

Chitosan Mechanism for Blood Clotting

Chitosan is a highly effective hemostatic agent, primarily due to its cationic properties, attributed to the positively charged NH_3^+ group (Gheorghită, Chen). Erythrocytes naturally carry a negative surface charge, which creates an electrostatic attraction between the two oppositely charged molecules. This interaction leads to the crosslinking of chitosan with erythrocytes, supporting the hypothesis that chitosan actively attracts erythrocytes (Gheorghită). As erythrocytes accumulate, a “mucoadhesive barrier” forms at the wound site, effectively halting bleeding (Chen). This barrier is composed of aggregated erythrocytes and proteins naturally present in blood plasma and surrounding tissues (Chen).

The proposed mechanism underlying this process involves the protonation state of chitosan. In its protonated form, chitosan carries a positive charge on its NH_3^+ group. However, as the pH increases upon exposure to blood, chitosan undergoes deprotonation, releasing positively charged H^+ ions into the surrounding environment (Chen). The negatively charged COO^- groups on the erythrocyte surface are attracted to these H^+ ions, reducing the overall surface charge of the erythrocytes (Chen). This reduction in repulsive forces between erythrocytes, without altering the attractive forces, facilitates aggregation and the subsequent formation of a cohesive layer of erythrocytes on the chitosan surface (Chen).

In addition to the formation of the erythrocyte layer, chitosan's slight acidity may contribute to local vasoconstriction. Its high absorptive capacity, combined with the application of pressure opposing blood flow, increases fluid viscosity, further supporting the formation of the mucoadhesive barrier (Chen).

Furthermore, some studies suggest that chitosan may induce platelet aggregation and adhesion, activating coagulation factors within classical coagulation pathways. However, other experimental findings indicate that chitosan's hemostatic properties are independent of these pathways (Chen, Thatte, Chou, Rou, Millner, Brown). These conflicting results highlight the need for further investigation into the precise mechanisms of chitosan-mediated hemostasis.

Chitosan Mechanism for Antimicrobial Activity

Chitosan has demonstrated a broad spectrum of antimicrobial activity. Previous studies have shown that chitosan inhibits the growth of various microorganisms, including yeast, bacteria, and fungi (Mirbagheri). Although the precise mechanisms underlying this antimicrobial property remain uncertain, several theories have been proposed (Yilmaz).

The most widely accepted theory attributes chitosan's antimicrobial effects to its cationic nature. The positively charged NH_3^+ groups in chitosan interact with the negatively charged bacterial cell wall (Nagy). This interaction disrupts the cell wall's integrity, altering membrane permeability, and ultimately leads to the leakage of intracellular components and cell lysis (Yilmaz, Nesovic, Sanmugan, Younes). The polycationic structure of chitosan is crucial for its antibacterial activity.

Chitosan typically exhibits a pKa above environmental pH, allowing it to remain predominantly in its protonated form. This enables electrostatic interactions between the positively charged

chitosan and the anionic surfaces of microorganisms (Kong). Notably, the pKa of chitosan can be modified through its derivatives or by combining it with other materials, enabling it to maintain its protonated state at higher pH values (Yang). The density of positive charges on chitosan correlates directly with its antibacterial strength, which is often enhanced by increasing the degree of deacetylation (DD). Higher DD levels result in the removal of acetyl groups, thereby increasing the number of positive charges and enhancing antibacterial efficacy (Yilmaz).

In addition to this primary mechanism, other theories have been proposed. One suggests that chitosan acts as a chelating agent, sequestering trace metal ions and inhibiting microbial growth through the disruption of toxin production (Nagy, Divya). Another hypothesis posits that at lower molecular weights, chitosan can penetrate bacterial cells, bind to DNA, and inhibit replication, ultimately causing cell death (Divya).

It is important to note that the antimicrobial effectiveness of chitosan varies depending on numerous intrinsic and extrinsic factors, including its degree of deacetylation, molecular weight, solubility, pH, temperature, and the specific microorganism being targeted (Younes).

Gelatin

Gelatin, derived from collagen—a natural protein abundant in the extracellular matrix (ECM) of animal tissues—exhibits numerous properties that make it an excellent candidate for tissue engineering applications. Due to its natural origin, gelatin demonstrates high biocompatibility, allowing it to integrate seamlessly into biological systems. This integration supports essential cellular processes such as adhesion, proliferation, and differentiation (Afewerki). As a collagen

derivative, gelatin closely mimics the structure of the body's native ECM, making it particularly conducive to cellular attachment and migration (Afewerki).

One of gelatin's most desirable attributes is its biodegradability. It can be enzymatically degraded within the body, ensuring that scaffolds made from gelatin naturally break down over time without producing toxic byproducts. This eliminates the need for surgical removal and reduces the risk of adverse immune responses, thereby enhancing the healing process. Furthermore, gelatin's hydrophilic nature allows for improved water retention and facilitates the diffusion of nutrients, oxygen, and waste—factors critical for maintaining cell viability and promoting tissue regeneration (Afewerki).

Despite these advantages, gelatin does have some limitations, including poor mechanical properties and rapid enzymatic degradation (Afewerki). However, these shortcomings can be addressed through crosslinking with other materials. By selecting appropriate crosslinkers with specific properties, gelatin's mechanical strength and elasticity can be significantly enhanced, making it a versatile and effective component in implantable medical devices. Additionally, gelatin is widely available and cost-effective, further supporting its utility as a consumer-friendly material for biomedical applications (Afewerki).

Chitosan-Gelatin Crosslinking: Overview of Common Crosslinkers

Structurally, both chitosan and gelatin possess free amine groups. To successfully link these biomaterials and enhance their suboptimal mechanical properties, the use of a crosslinker is essential. Various crosslinking agents are available, each offering distinct advantages and

disadvantages. The following sections provide an overview of several commonly used crosslinkers, discussing their benefits and limitations.

Genipin

Genipin is a natural crosslinker derived as a colorless aglycone extract from the genipap fruit (*Genipa americana*) (Wang). It reacts effectively with the free amine groups in chitosan and gelatin (Wang), making it a favorable choice for biomedical applications. Key advantages of genipin include its low toxicity, biocompatibility with human cells, and ability to improve mechanical strength, elasticity, and wound healing through antioxidant and anti-inflammatory properties (Utami). However, genipin has notable drawbacks, including its relatively high cost compared to synthetic crosslinkers and a slower reaction time during the crosslinking process (Nair, Sapula).

Glutaraldehyde

Glutaraldehyde is a widely used synthetic crosslinker in scaffold production due to its strong ability to link free amine groups (Yang 2018). This crosslinking capacity enhances the mechanical strength and structural integrity of scaffolds (Yang 2018). Additionally, its crosslinking degree can be adjusted by modifying the concentration and reaction time, allowing tailored mechanical and degradation properties (Yang 2018). Glutaraldehyde is cost-effective and readily available, making it suitable for large-scale applications (Yang 2018). Despite these advantages, glutaraldehyde poses cytotoxicity risks, potentially harming surrounding tissues and cells. To improve its biocompatibility, additional processing steps are required, which increase time and cost (Yang 2018).

Water-Soluble Carbodiimides – EDC/NHS

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), often used in combination with *N*-

hydroxysuccinimide (NHS), is a versatile crosslinker capable of forming bonds between the free amine groups of chitosan and the carboxyl groups of gelatin. This crosslinking enhances the mechanical strength and elasticity of the resulting polymer network, improving its resistance to deformation under stress (Hutomo). EDC also reduces gelatin solubility, enhancing scaffold stability and biocompatibility without compromising hydrophilicity, though it slightly lowers the swelling ratio (Hutomo, Xing). However, EDC does not integrate into the crosslinked network, resulting in zero-length crosslinking, which can reduce overall mechanical strength (Islam). Moreover, optimal reaction conditions (e.g., pH, concentration, and reaction time) are critical, as suboptimal conditions can lead to incomplete crosslinking and undesired properties (Islam).

Tannic Acid

Tannic acid, a plant-derived polyphenol, functions as a natural crosslinker through hydrogen, ionic, and covalent bonds, as well as hydrophobic interactions (Sapula). Its use enhances scaffold mechanical properties and resistance to enzymatic degradation (Sapula). Additionally, tannic acid offers several bioactive benefits, including antimicrobial, anti-cancer, anti-inflammatory, hemostatic, and oxidizing properties, which contribute to wound healing and infection prevention (Sapula). However, at higher concentrations, tannic acid can exhibit cytotoxic effects, potentially harming surrounding cells (Wang 2019).

Transglutaminase

Transglutaminase is an enzymatic crosslinker with high specificity for the amine groups in gelatin (Bertoni). It provides desirable qualities such as improved mechanical strength, biocompatibility, and non-toxicity (Bertoni). However, its application is limited by its higher cost compared to chemical crosslinkers and its reduced efficiency in crosslinking chitosan (Bertoni).

Procedure

Various methods are commonly employed to fabricate scaffolds, but electrospinning offers distinct advantages. This technique provides fine control over the morphology of the produced fibers, including their thickness, porosity, structure, and diameter. These parameters can be precisely adjusted by varying the solution viscosity, voltage, and collector type (Flores). Such control is crucial for mimicking the extracellular matrix (ECM), which is essential in tissue engineering. Electrospinning also results in a high surface area, promoting improved cell attachment, proliferation, and nutrient exchange (Flores). Additionally, this method facilitates the blending of multiple polymers and the incorporation of bioactive materials into the scaffolds. In contrast, freeze-drying has some limitations, such as the absence of a fibrous structure (lacking ECM-like architecture), reduced mechanical properties, lower surface area, and a slower processing time (Capuana). While freeze-drying allows for larger pore sizes and is simpler to fabricate, electrospinning is preferred due to its superior properties for scaffold production.

Materials

Gelatin powder (type A or B), genipin powder, acetic acid, and medium molecular weight chitosan, deionized water

Preparation

To prepare the solution, dissolve chitosan in 1-2% (v/v) acetic acid at a concentration of 1-5% (w/v), and stir with a magnetic stirrer at room temperature until fully dissolved. Next, dissolve gelatin powder in deionized water at room temperature to a concentration of 10-20% (w/v), stirring until fully dissolved. Combine both solutions to achieve the desired gelatin-to-chitosan

ratio, and continue stirring in an ultrasonic bath until the solutions are fully integrated (the time will vary depending on the solution size). Incubate the solution at 37°C for 3 hours to prepare for electrospinning. Electrospinning conditions can be adjusted to achieve specific properties; however, previous studies have employed the following conditions: 25°C, 45% humidity, a 30 G needle, a flow rate of 0.3 mL/h, a voltage of 18 kV, and a nozzle-to-collector distance of 15 cm (Khalilimofrad).

After the initial electrospinning process, crosslinking with genipin is performed. Dissolve genipin in deionized water to achieve a concentration of 0.1%-1% (w/v). Submerge the chitosan-gelatin scaffold in a shallow container and add the genipin solution until the scaffold is fully submerged. Maintain room temperature and incubate the scaffold for 12-48 hours, with longer incubation times resulting in a higher degree of crosslinking. After the crosslinking reaction is complete, wash the scaffold 3-5 times with deionized water to remove residual genipin. Allow the scaffold to air dry thoroughly.

Conclusion

The proposed chitosan-gelatin scaffold has the potential to significantly advance modern medicine. Structurally, this scaffold could be applied both internally, in areas where gauze is typically used, and externally, where bandages are commonly employed. Moreover, the scaffold is designed to possess key properties, including antifungal, antimicrobial, hemostatic, biodegradable, and biocompatible characteristics, making it versatile for various medical applications. These properties could enhance surgical outcomes by preventing excessive bleeding, reducing the risk of infection, and promoting the healing and regeneration of multiple

tissue types. Potential applications include the regeneration of skin, nerve, muscle, cartilage, salivary glands, blood vessels, adipose tissue, ligaments, and bone (Rodriguez). Advancing this line of research could revolutionize modern medicine, serving as a catalyst for making tissue regeneration a viable solution for a broad patient population.

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