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Collagen Synthesis During Wound Repair in Infected Albino Rats Using Ciprofloxacin-Loaded Gelatin Microspheres Incorporated into a Collagen Scaffold: A Histological Approach Using Masson's Trichrome Staining

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Abstract: The production of collagen fibers is essential for wound healing because it creates a scaffold of collagen on which new cells may move and proliferate. It indicates cellular activity and improves strength and flexibility. Collagen synthesis is influenced by a number of factors, including age, health, infection, and diet. Collagen dressings often have the ability to increase the production of collagen. Collagen production can be adversely affected by infected cutaneous wounds, which can result in a delayed healing process, a higher risk of scarring, weakened tissue, and functional impairment. For this reason, in order to speed up wound healing, antimicrobial agents have been added to the collagen scaffold. Furthermore, the prolonged delivery of antimicrobial agent-loaded gelatin microspheres to infected wounds promotes quicker wound healing and effective collagen synthesis in the granulated tissue. These scaffolds may make superior wound dressings in clinical settings in light of this relationship.

Keywords: Type 1 collagen; Collagen dressings; Porous scaffolds; Gelatin microspheres; Masson's trichrome staining

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1. Introduction

Wound healing and regeneration are complex biological processes that occur in response to tissue injury. The ultimate goal of wound healing is to restore the structure and function of damaged tissues. The process of wound healing involves a series of overlapping phases, including hemostasis, inflammation, proliferation, and remodeling. During the hemostasis phase, blood vessels constrict to reduce blood loss, and platelets form a clot to stop bleeding. In the inflammation phase, immune cells are recruited to the wound site to remove debris

and fight off infection. This phase is characterized by redness, swelling, and heat. The proliferation phase is characterized by the formation of new tissue, including the growth of new blood vessels and the production of extracellular matrix. This phase also involves the migration and proliferation of various cell types, such as fibroblasts and keratinocytes, to help rebuild the damaged tissue. The final phase of wound healing is remodeling, during which the newly formed tissue is remodeled and strengthened. This phase can last for months or even years, as the tissue gradually gains strength and flexibility. In some cases, particularly in lower organisms such as amphibians and certain invertebrates, wound healing can also involve regeneration, which is the process of replacing damaged or lost tissues with new functional tissue. This process often involves the dedifferentiation of nearby cells, which then proliferate and differentiate to replace the lost tissue. Research into wound healing and regeneration is ongoing, and scientists are exploring new ways to enhance these processes, such as through the use of stem cells, growth factors, and tissue engineering techniques. Understanding the intricacies of wound healing and regeneration is crucial for developing new therapies to improve outcomes for patients with injuries and chronic wounds [1,2].

Collagen is a key component of the extracellular matrix and plays a crucial role in wound repair. During the process of wound healing, collagen undergoes degradation and synthesis to facilitate tissue regeneration. Collagen degradation occurs during the initial inflammatory phase of wound repair. This process is mediated by various enzymes, such as matrix metalloproteinases (MMPs) and collagenases, which break down the damaged collagen fibers at the wound site. This degradation of collagen helps to clear the way for new tissue formation and remodeling. Once the initial inflammatory phase is complete, the synthesis of new collagen takes place during the proliferative phase of wound repair. Fibroblasts, which are specialized cells responsible for producing collagen, migrate to the wound site and begin synthesizing new collagen fibers. This newly synthesized collagen helps to strengthen the wound and promote tissue regeneration. The balance between collagen degradation and synthesis is essential for proper wound healing. Excessive collagen degradation can lead to delayed wound healing and chronic wounds, while excessive collagen synthesis can result in the formation of scar tissue. Therefore, the regulation of collagen degradation and synthesis is critical for successful wound repair. Various factors, such as growth factors, cytokines, and mechanical stimuli, regulate collagen degradation and synthesis during wound repair. Understanding the mechanisms involved in collagen turnover during wound healing is essential for developing new therapeutic approaches to promote efficient tissue regeneration and minimize scar formation [3,4].

Collagen biomaterials have been widely investigated and used in the field of wound repair and regeneration due to their biocompatibility, biodegradability, and ability to mimic the natural extracellular matrix. Collagen is the main structural protein in the extracellular matrix of various tissues, including skin, and plays a crucial role in wound healing processes such as cell migration, proliferation, and tissue remodeling. In the context of wound repair and regeneration, collagen biomaterials can be used in various forms, including sponges, sheets, gels, and powders, to provide a scaffold for cell attachment, migration, and proliferation. These biomaterials can also be functionalized with growth factors, antimicrobial agents, or other bioactive molecules to enhance their therapeutic potential. Collagen biomaterials have been shown to promote wound healing by facilitating the formation of new blood vessels, promoting the deposition of new extracellular matrix, and reducing scar formation. Additionally, they have been found to have antimicrobial properties, which can help prevent infection in chronic wounds. Furthermore, collagen biomaterials have been used in combination with other therapeutic approaches, such as stem cell therapy and tissue engineering, to enhance their regenerative potential. For example, collagen-based scaffolds can be seeded with stem cells or other therapeutic cells to enhance their ability to promote tissue regeneration. Overall, collagen biomaterials hold great promise for wound repair

and regeneration and continue to be an area of active research and development in the field of regenerative medicine. Their biocompatibility, biodegradability, and ability to mimic the natural extracellular matrix make them a promising option for promoting wound healing and tissue regeneration [5-7].

This paper reveals the collagen synthesis during soft tissue regeneration of infected wound repair and regeneration through the collagen biomaterials applied on the wound surface. In addition to that, Masson's trichrome (M&T) staining of granulated tissue investigates collagen synthesis in wound surfaces.

2. Materials and methods

2.1. Type 1 collagen

Type 1 collagen was extracted from bovine tendons as per the reported protocol in the scientific literature. Type 1 collagen was used as a base biomaterial for the fabrication of wound-healing biomaterial scaffolds [8].

2.2. Preparation of gelatin microspheres

Gelatin microspheres were made with 7.5 weight percent ciprofloxacin diluted in 10 ml of aqueous phase using the water-in-oil emulsion technique. To insert the gelatin microspheres into the collagen scaffold, a predetermined amount of collagen solution was mixed with 0.5 g of ciprofloxacin-loaded gelatin microspheres using a standard procedure [9].

2.3. Preparation of collagen scaffolds

Once the purified collagen was obtained, it was used to fabricate a scaffold. This is typically done by mixing the collagen with a buffer solution and then casting it into a mold of the desired shape. The scaffold may also be cross-linked using methods such as chemical cross-linking or ultraviolet irradiation to improve its stability and mechanical properties. The fabricated scaffold was then characterized to assess its physical, mechanical, and biological properties. This may involve testing for porosity, pore size, mechanical strength, and biocompatibility. Before the scaffold can be used for tissue engineering applications, it must be sterilized to remove any potential contaminants. This can be achieved using methods such as gamma irradiation, ethylene oxide treatment, or autoclaving. Overall, the fabrication of scaffolds from type 1 collagen from bovine tendons involved a series of steps to extract, purify, fabricate, characterize, and sterilize the collagen-based scaffold for use in tissue engineering and regenerative medicine applications [10,11].

2.4. Preparation of gelatin microspheres-impregnated collagen (GMC) scaffolds

The drug-loaded gelatin microspheres were added into the type 1 collagen solution and mixed well. Then, the suspension was cast into the plastic trough to fabricate as microspheres-impregnated collagen scaffolds. As discussed above, the given scaffolds were sterilized using an ethylene oxide sterilization process [9].

2.5. *In vivo* studies

Male Wister albino rats weighing between 150 and 200 g were used in this study. The animals were fed commercial pellet food (Hindustan Lever, Bangalore, India) and had unlimited access to water. The animal experiment was authorized by the Institute's ethical committee, which also offered guidelines for its execution. After the wound was made, the experimental animals were clothed with a collagen scaffold with an antibiotic, a collagen scaffold without any design, and a collagen scaffold with plain collagen, whereas the control group was given a gauze dressing. All of the rats were given daily changes, and the antibiotic and specially-made bandages were replaced every two days [10,11].

2.6. Masson's trichrome staining

Masson's trichrome staining is a histological staining technique used to visualize connective tissue, such as collagen and muscle fibers, in tissue samples. The staining method involves using three different dyes to highlight different components of the tissue. The first dye, Weigert's iron hematoxylin, stains cell nuclei and muscle fibers blue-black. The second dye, Biebrich scarlet-acid fuchsin, stains cytoplasm and muscle fibers red. The third dye, aniline blue, stains collagen and smooth muscle fibers blue. The combination of these three dyes allows for the visualization of different tissue components, making it useful for studying fibrosis, muscle pathology, and other connective tissue disorders. Masson's trichrome staining is commonly used in research and clinical pathology to examine tissue samples from biopsies or surgical specimens. In our current study, the M&T stains on the granulated tissue from wounds were performed to evaluate the collagen content in the tissues [9].

3. Results and discussion

3.1. Collagen synthesis

Collagen synthesis is a critical component of wound healing, playing a key role in forming the new tissue that repairs the damage. Collagen is the most abundant protein in the human body, forming the structural foundation of various tissues, including skin, tendons, and bones. During wound healing, its synthesis is crucial for several reasons. It provides a scaffold as collagen fibers create a supportive matrix for new cells to migrate and proliferate, filling the wound gap. It enhances strength and flexibility, as collagen fibers intertwine and mature, they provide tensile strength and elasticity to the newly formed tissue, restoring its functionality. It also signals cellular activity, collagen fragments released during tissue breakdown interact with cells, stimulating their growth and collagen production [3].

The process of collagen synthesis in wound healing follows a well-coordinated sequence. (1) Inflammatory phase: In the initial inflammatory stage, immune cells like macrophages clear debris and release growth factors that activate fibroblasts, the primary collagen-producing cells. (2) **Proliferation phase:** Activated fibroblasts migrate to the wound site and begin producing procollagen, an immature precursor molecule. Procollagen undergoes modifications and assembles into collagen fibers within the fibroblast. (3) **Maturation and remodeling:** Newly formed collagen fibers are secreted into the extracellular space, where they mature and cross-link with each other, gaining strength and stability. This phase can last for weeks or even months [12].

Several factors can influence the rate and quality of collagen synthesis during wound healing, including: (1) Nutrition: Adequate intake of protein, vitamin C, and other essential nutrients provides the building blocks for collagen production. (2) Age: Collagen production naturally declines with age, potentially impacting wound healing efficiency. (3) Underlying medical conditions: Chronic diseases like diabetes or malnutrition can impair collagen synthesis. (4) Infection: The presence of infection can hinder the healing process and collagen synthesis. Maintaining a healthy lifestyle with a balanced diet, regular exercise, and proper wound care can promote optimal collagen synthesis for efficient wound healing. Additionally, certain therapies like topical vitamin C application or collagen dressings are being explored to support collagen production in wound management. By understanding the intricate role of collagen synthesis in wound healing, we can appreciate its significance in tissue repair and explore avenues to optimize this process for better healing outcomes.

3.2. Impaired collagen synthesis

Infected dermal wounds lack collagen synthesis. Infections in dermal wounds significantly affect collagen synthesis and negatively impact the overall healing process, described as follows. (1) Direct damage: Pathogens

can directly damage fibroblasts, the cells responsible for collagen production, reducing their number and function. (2) Inflammatory disruption: The prolonged inflammatory response triggered by infection hinders the orderly progression of wound healing phases, impacting collagen synthesis and maturation. (3) Nutrient depletion: Bacteria compete with host cells for essential nutrients like amino acids and vitamins, crucial for collagen production. (4) Protease activity: Many pathogens produce proteases, enzymes that break down proteins, including collagen, further compromising tissue strength and integrity. (5) Cytokine imbalance: The infection-induced release of certain inflammatory cytokines can suppress collagen synthesis while promoting collagen degradation [13].

The consequences of impaired collagen synthesis are as follows. Delayed wound closure: Reduced collagen production slows down the formation of granulation tissue and re-epithelialization, delaying wound closure. Increased risk of scarring: Inadequate collagen synthesis and disorganized fibers can lead to excessive scar formation. Reduced tissue strength: Insufficient collagen weakens the repaired tissue, making it more susceptible to further damage. Functional impairment: Depending on the wound location, impaired collagen synthesis might affect functionality and movement. Prompt diagnosis and treatment of infection are crucial to minimize its impact on collagen synthesis and promote optimal wound healing. This often involves the following: (1) Antibiotics: Selecting the appropriate antibiotic based on the identified pathogen is essential for eliminating the infection. (2) Debridement: Removing dead tissue and foreign debris can reduce the bacterial load and create a favorable environment for healing. (3) Wound care: Maintaining a clean and moist wound environment promotes healing and prevents further infection.

Infected dermal wounds pose a significant challenge for healthcare professionals, as the presence of bacteria can impede the healing process and lead to further complications. In recent years, there has been growing interest in developing innovative approaches to combat this issue, and one promising solution is the use of antibiotic-incorporated collagen matrices. Collagen is a natural protein found in the extracellular matrix of connective tissues, and it plays a crucial role in wound healing by providing structural support and promoting cell migration and proliferation. By incorporating antibiotics into collagen matrices, researchers aim to create a localized and sustained release of antimicrobial agents directly at the wound site, effectively targeting and eradicating the infection while also providing a supportive environment for tissue regeneration. The use of antibiotic-incorporated collagen matrices offers several advantages for infected dermal wound healing. Firstly, the controlled release of antibiotics from the matrix can help maintain therapeutic levels at the wound site, ensuring continuous protection against bacterial colonization. This targeted approach minimizes systemic exposure to antibiotics, reducing the risk of antibiotic resistance and adverse effects. Additionally, the collagen matrix itself serves as a scaffold for tissue repair, promoting the formation of new blood vessels and supporting the ingrowth of fibroblasts and keratinocytes. This dual functionality of the matrix not only addresses the infection but also accelerates the overall healing process, leading to improved outcomes for patients with infected dermal wounds. Several studies have demonstrated the efficacy of antibiotic-incorporated collagen matrices in promoting the healing of infected dermal wounds. These matrices have been shown to effectively reduce bacterial load, improve tissue regeneration, and enhance overall wound healing rates. As a result, they hold great promise for clinical applications in the management of infected dermal wounds, offering a targeted and comprehensive approach to addressing this challenging clinical problem. In short, antibiotic-incorporated collagen matrices represent an innovative and promising strategy for the treatment of infected dermal wounds. By providing localized antimicrobial therapy and supporting tissue regeneration, these matrices offer a comprehensive solution for healthcare professionals seeking to improve outcomes for patients with infected wounds. Continued research and development in this area hold great potential for advancing the field of wound

care and enhancing the quality of life for individuals with challenging wound-healing needs [14].

3.3. Collagen scaffolds

Figures 1 and **2** show the plain collagen scaffold and drug-loaded microspheres-impregnated collagen scaffold for the treatment of infected dermal wounds. Normally, a pure collagen scaffold was not fit for the treatment of infected dermal wounds as collagen is a fibrous protein in nature and is highly susceptible to microbial degradation via enzymatic processes. Therefore, antimicrobials have been impregnated into the collagen matrices to combat wound pathogens and provide sustained release to the wound surface. To provide efficient controlled release of antimicrobials into the wound surface, drug-loaded microspheres were impregnated into the collagen scaffolds. **Figure 3** shows the drug-loaded gelatin microspheres.

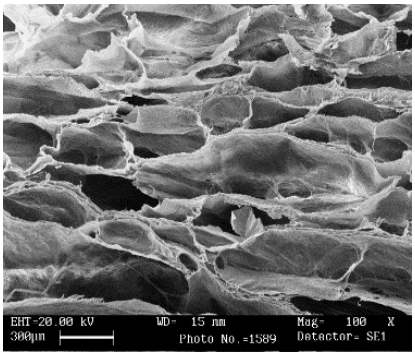

Figure 1. Porous collagen scaffolds

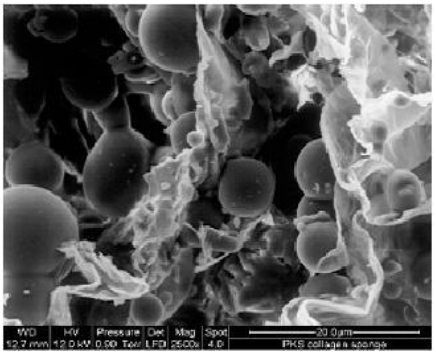

Figure 2. Drug-loaded microspheres-impregnated collagen scaffolds

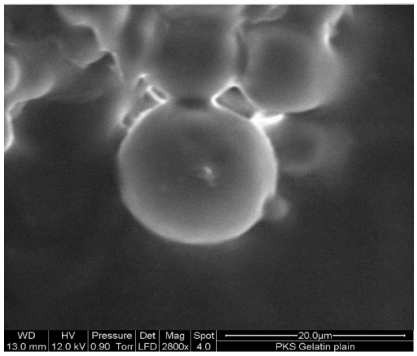

Figure 3. Drug-loaded gelatin microspheres

Drug-loaded microspheres incorporated into collagen matrices have shown promise in the treatment of infected dermal wounds. The microspheres can be loaded with antibiotics or other antimicrobial agents, which are then released slowly and continuously into the wound site, providing sustained antimicrobial activity. The collagen matrices provide a scaffold for the microspheres, promoting wound healing and tissue regeneration. Collagen is a natural component of the extracellular matrix and has been shown to support cell migration, proliferation, and differentiation, as well as promote angiogenesis and tissue remodeling. When incorporated into collagen matrices, the drug-loaded microspheres can effectively target and eliminate the infectious microorganisms present in the wound, while also promoting the healing process. This combination therapy has the potential to improve the outcomes of infected dermal wound healing, reducing the risk of chronic wounds, and minimizing the need for systemic antibiotics. Overall, drug-loaded microspheres incorporated into collagen matrices represent a promising approach for the treatment of infected dermal wounds, offering targeted antimicrobial therapy and support for tissue regeneration. Further research and clinical trials are needed to fully evaluate the efficacy and safety of this approach in clinical settings.

3.4. Role of collagen synthesis in wound contraction

Collagen synthesis plays a crucial role in wound contraction, which is an essential step in the wound-healing process. When there is a wound, the body initiates a series of events to repair the damaged tissue. One of these events is the synthesis of collagen, a structural protein that provides strength and support to the skin and other connective tissues. During the wound-healing process, fibroblasts, a type of cell found in connective tissue, migrate to the site of the wound and begin synthesizing collagen. As the collagen fibers accumulate at the wound site, they form a scaffold that helps to support the growth of new tissue and facilitate the contraction of the wound. This contraction reduces the size of the wound, bringing the edges of the skin closer together and

promoting the formation of a stronger and more functional scar. In addition to providing structural support, collagen also plays a role in signaling other cells involved in the wound-healing process, such as immune cells and blood vessels, to coordinate their activities and facilitate the repair of the damaged tissue. Overall, collagen synthesis is essential for wound contraction and the overall healing of a wound. It helps to restore the integrity of the skin and promote the formation of a functional scar, ultimately restoring the barrier function of the skin and protecting the body from infection and further injury [15].

These collagen scaffolds are made quickly and efficiently by using the described technology. The group receiving scaffold treatment saw a quicker rate of wound healing in comparison to untreated wounds. It was shown that the risk of infections is significantly lower and that 12 days were needed for complete soft tissue regeneration. M&T staining confirmed the perfect regeneration of the dermis and epidermis in the treated groups by showing the production of well-deposited collagen bundles in those groups.

3.5. Results of Masson's trichrome staining

M&T staining of granulated tissue reveals the insoluble collagen in tissue. It can be used to visualize and distinguish between collagen, muscle fibers, and other components of the tissue. In the case of infected dermal wounds, it can be used to assess the extent of tissue damage, the presence of inflammatory cells, and the organization of the granulation tissue. It can also provide information on the deposition of collagen and the overall healing process of the wound. It can be a valuable tool in the histological analysis of granulated tissue from infected dermal wounds, providing important insights into the composition and structure of the tissue, and aiding in the assessment of the wound-healing process.

Figure 4 shows the M&T staining of granulated tissue taken from the wound on the 4th day. The open wound group shows neutrophils on the granulated tissue due to the microbial infection on the wound surface. There was no clear formation of dermis and epidermis on the wound. There was no collagen synthesis in this group due to microbial pathogens on the wound surface. In the case of antimicrobial agents incorporated-collagen scaffolds and gelatin microspheres-impregnated collagen scaffolds, the neutrophils count was considerably decreased and the onset of collagen biosynthesis in the granulated tissue increased. The bluish color indicates that collagen fibers in the granulated tissue were observed. **Figure 5** shows the M&T staining of granulated tissue taken from the wound on the 8th day. There was a reduction of neutrophils in the granulated tissue taken from antimicrobial agents incorporated-collagen scaffold (AMC) and microspheres incorporated-collagen (GMC) scaffolds and the regeneration of the dermis and epidermis has been started effectively. In the case of the open wound group, the dermis and epidermis have poorly regenerated, and there are neutrophils present on the wound surface. **Figures 6** and **7** show the M&T staining of granulated tissue taken from the wound on the 12th and 16th day. The wound was better regenerated by AMC and GMC scaffolds than that of the open wound group.

When contrasting the treatment group (ciprofloxacin-loaded gelatin microsphere-impregnated collagen scaffold) with the control group (open wound, ciprofloxacin-incorporated collagen scaffold), the treatment group exhibited the formation of collagen bundles as well as well-formed dermis and epidermis. Similarly, the group that had GMC treatment showed well-formed collagen bundles, as confirmed by M&T staining of granulated tissue. In addition, compared to other groups, the GMC group's dermis and epidermis demonstrated excellent regeneration and full wound healing. In addition, in comparison to other groups, the GMC scaffold entirely eliminated wound pathogens on the 12th day of wound healing and efficiently managed the bacterial load at the wound site.

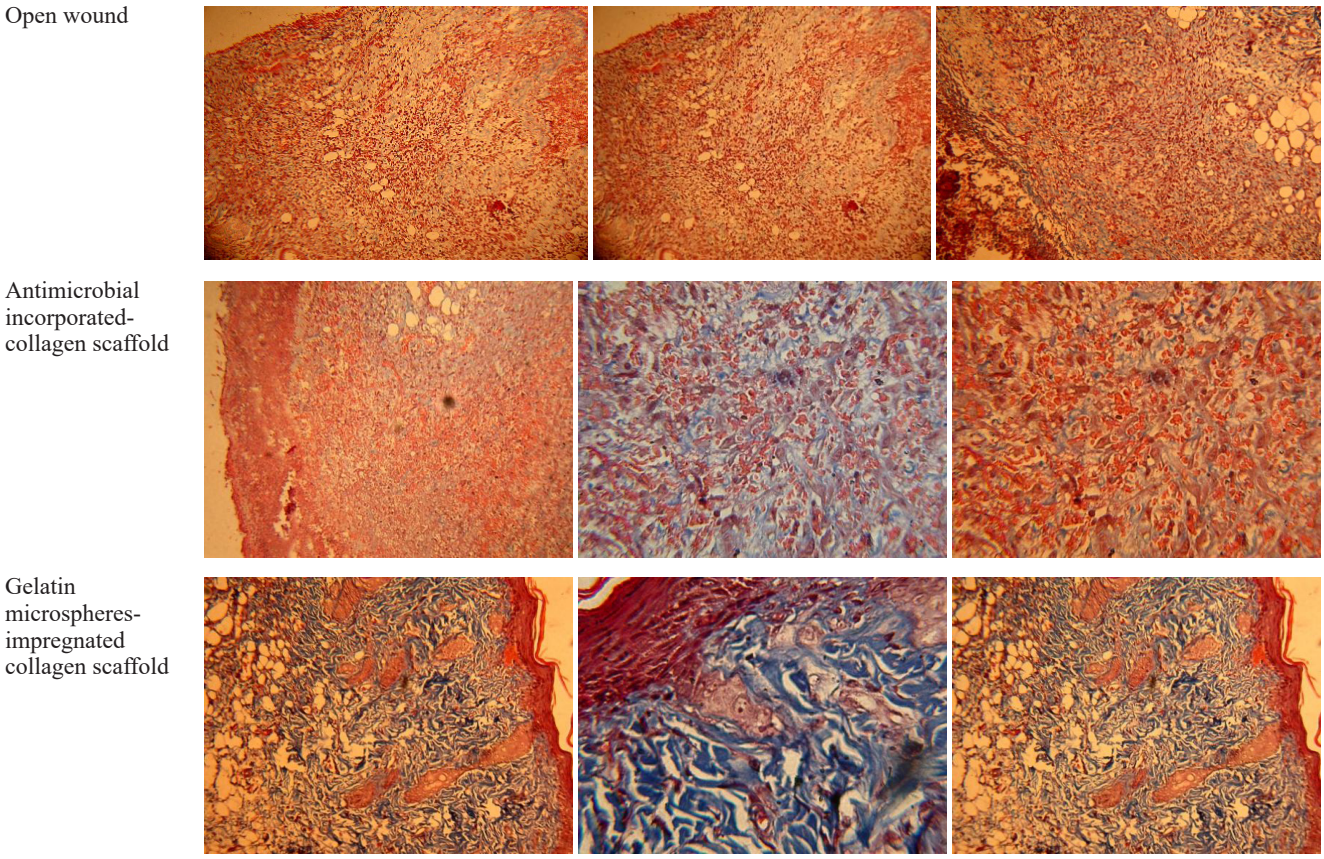

Figure 4. M&T staining of granulated tissue taken on the 4th day of wound repair

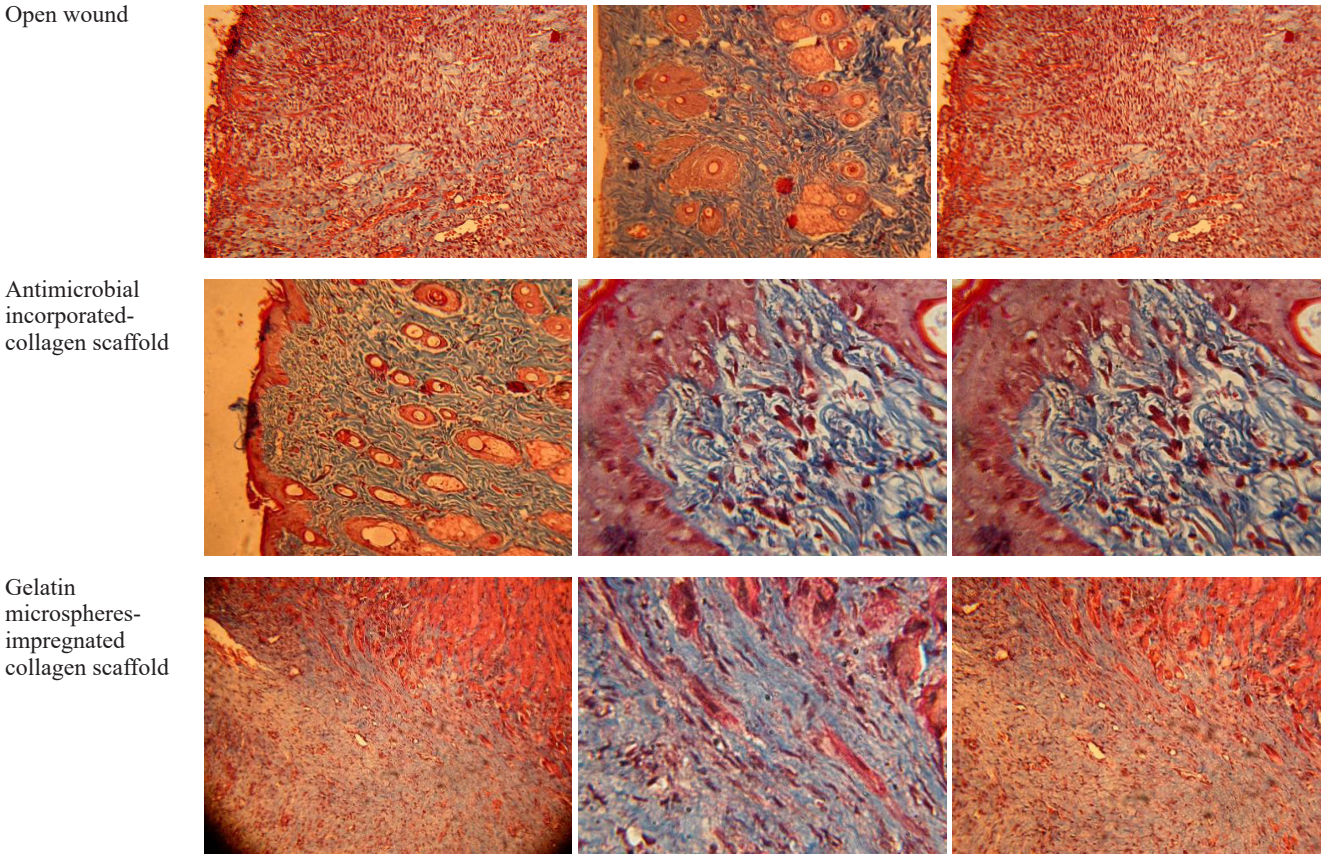

Figure 5. M&T staining of granulated tissue taken on the 8th day of wound repair

Open wound

Antimicrobial incorporated-collagen scaffold

Gelatin microspheres-impregnated collagen scaffold

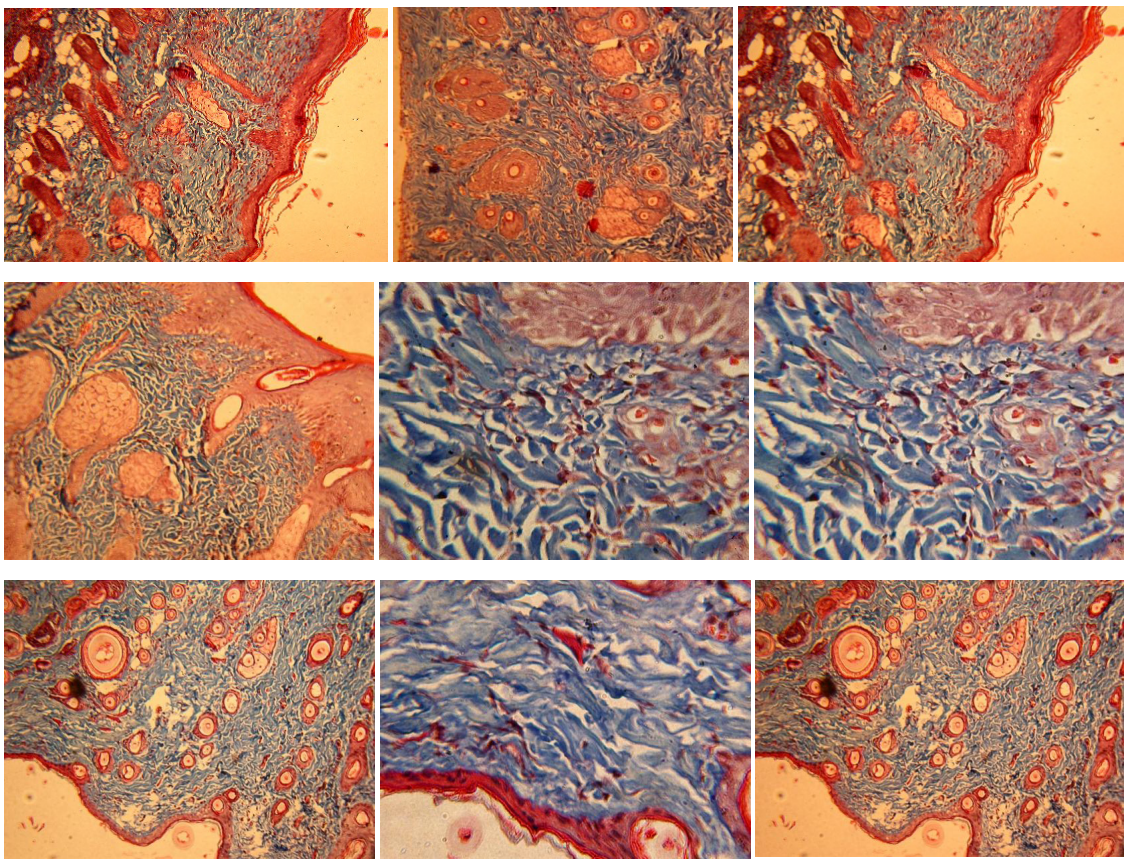

Figure 6. M&T staining of granulated tissue taken on the 12th day of wound repair

Open wound

Antimicrobial incorporated-collagen scaffold

Gelatin microspheres-impregnated collagen scaffold

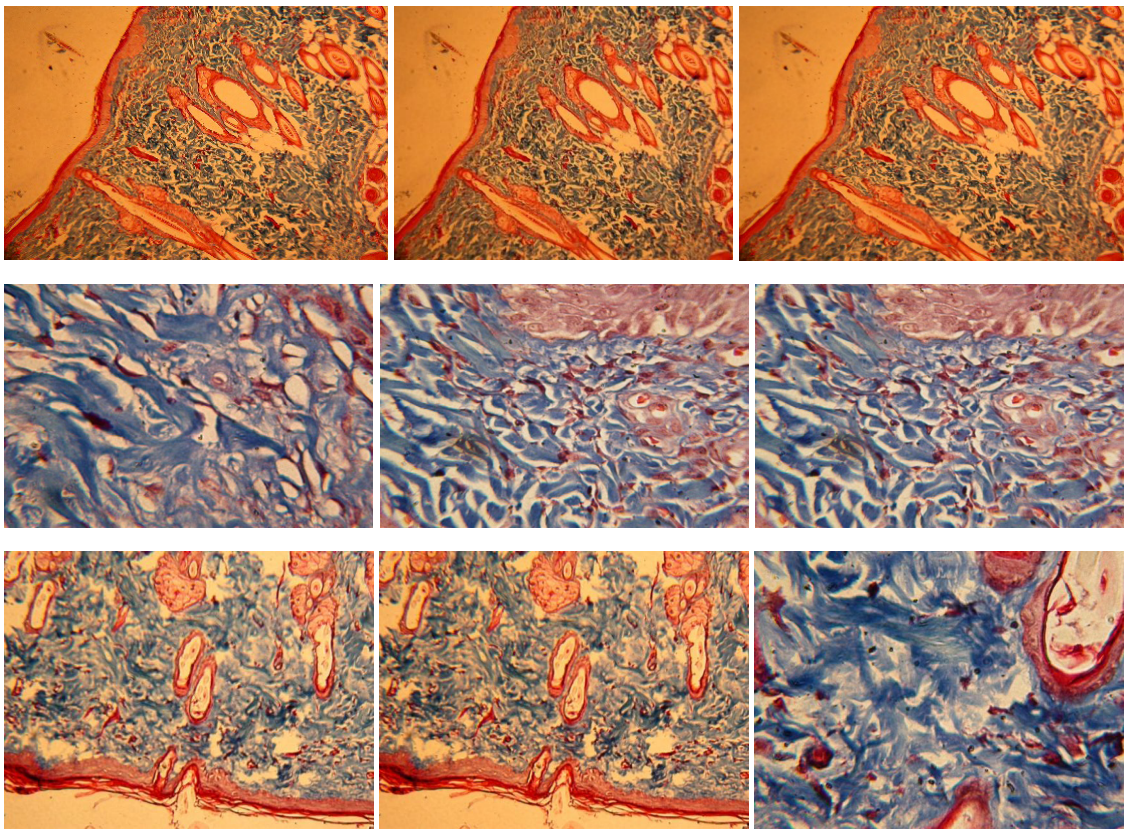

Figure 7. M&T staining of granulated tissue taken on the 16th day of wound repair

Collagen biomaterials have been shown to have a positive effect on collagen synthesis in wound repair and regulation. When applied to a wound, collagen biomaterials can provide a scaffold for new collagen formation, promoting the body's natural healing process. Studies have demonstrated that collagen biomaterials can stimulate the production of new collagen by fibroblasts, the cells responsible for synthesizing collagen in the body. This can lead to improved wound healing and tissue regeneration. In addition, collagen biomaterials can also regulate the synthesis of collagen by modulating the activity of enzymes and growth factors involved in collagen production. This can help to ensure that the newly formed collagen is organized and functional, contributing to the strength and integrity of the repaired tissue. Overall, the use of collagen biomaterials in wound repair has been shown to support and enhance the body's natural collagen synthesis processes, leading to improved outcomes in wound healing and tissue regeneration. The pure and plain collagen scaffold has the following limitations:

(1) Risk of infection: Collagen biomaterials can provide a favorable environment for bacterial growth, leading to an increased risk of infection in the wound.

(2) Delayed healing: In infected dermal wounds, the presence of collagen biomaterials may hinder the natural healing process, leading to delayed wound closure and increased inflammation.

(3) Allergic reactions: Some individuals may be allergic to collagen, leading to adverse reactions such as itching, redness, and swelling at the wound site.

(4) Poor wound drainage: Collagen biomaterials can impede proper wound drainage, leading to the accumulation of exudate and potentially exacerbating the infection.

(5) Inconsistent efficacy: The effectiveness of collagen biomaterials in infected dermal wounds may vary depending on the severity and type of infection, leading to inconsistent treatment outcomes.

(6) High cost: Collagen biomaterials can be expensive, and their use in the treatment of infected dermal wounds may not always be cost-effective, especially if the desired therapeutic effects are not achieved.

(7) Regulatory concerns: There may be regulatory concerns related to the use of collagen biomaterials in infected dermal wounds, particularly in terms of safety and efficacy. This can present challenges for healthcare providers and patients seeking treatment options.

4. Conclusion

Collagen synthesis is crucial for wound healing, providing a collagen scaffold for new cells to migrate and proliferate. It enhances strength and flexibility and signals cellular activity. Factors influencing collagen synthesis include nutrition, age, medical conditions, and infection. Normally, collagen dressings can promote optimal collagen synthesis. Infected dermal wounds can negatively impact collagen synthesis, leading to delayed wound healing, increased scarring risk, reduced tissue strength, and functional impairment. Therefore, antimicrobial agents have been incorporated into the collagen scaffold to accelerate wound repair. Furthermore, antimicrobial agent-loaded gelatin microspheres provide sustained release to the infected wounds resulting in faster wound healing and efficient collagen synthesis in the granulated tissue. Thus, these scaffolds could be better wound dressings for clinical applications.

Disclosure statement

The authors declare no conflict of interest.

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