

# Tissue Regeneration in Infected Wounds of Albino Rats Using Ciprofloxacin-Loaded Gelatin Microspheres Incorporated Collagen Scaffold: A Histological Approach with H&E Staining

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**Abstract:** A wound care system consisting of ciprofloxacin-loaded gelatin microspheres impregnated in a macroporous collagen scaffold was created to effectively control wound infection and regenerate soft tissue at the wound site. Histological and biochemical alterations were observed in infected wounds treated with these scaffolds in Albino Wistar rats. Furthermore, the study examined the immediate and prolonged release of ciprofloxacin from the scaffolds, as well as their function in eliminating bacterial infections and expediting the process of skin healing and regeneration. The developed technique was followed in the streamlined process of creating these collagen scaffolds. Compared to untreated wounds, the group receiving scaffold treatment experienced a faster rate of wound closure. It was noted that the rate of infections was considerably reduced and that full soft tissue regeneration occurred within 12 days. The development of well-deposited collagen bundles in the treated groups was demonstrated by H&E staining, which verified the flawless regeneration of the dermis and epidermis. The antimicrobial agent-loaded gelatin microspheres impregnated into the porous collagen scaffold demonstrated remarkable soft tissue regeneration and efficient infection control at the wound site.

**Keywords:** Gelatin microspheres; Collagen; Controlled release; Wound healing

**Online publication:** June 24, 2024

## 1. Introduction

Wound repair and regeneration is a complex physiological process that occurs in response to tissue damage. It involves a series of well-orchestrated events aimed at restoring the integrity and function of the injured tissue. In this article, we will delve into the intricacies of wound repair and regeneration, exploring the different phases and mechanisms involved. Wound repair is a dynamic process comprising four overlapping phases: hemostasis, inflammation, proliferation, and remodeling. Each phase plays a crucial role in facilitating the healing process.

- (1) Hemostasis: This initial phase involves the constriction of blood vessels and the formation of a blood clot to prevent excessive bleeding. Platelets and clotting factors play significant roles in this process.

- (2) **Inflammation:** Inflammation is the body's response to injury and is characterized by the migration of immune cells to the site of injury. These cells release various chemicals and growth factors that promote tissue repair and remove dead or damaged cells.
- (3) **Proliferation:** During the proliferation phase, new blood vessels are formed (angiogenesis), and fibroblasts produce collagen to replace the damaged tissue. Epithelial cells also migrate and divide to cover the wound surface.
- (4) **Remodeling:** The final phase of wound repair involves the maturation and remodeling of the newly formed tissue. Collagen fibers are reorganized, and the wound gradually gains strength. This phase can last for several months, and the final scar is often less noticeable.

In certain tissues, such as the liver and the epithelial cells of the skin, wounds can regenerate without leaving scars. This remarkable ability is attributed to the presence of stem cells that can differentiate and replace damaged cells. However, in most tissues, wound healing leads to scar formation. The reason behind scar formation lies in the reparative nature of wound healing. During wound repair, a specialized connective tissue called granulation tissue forms to bridge the gap and facilitate the regeneration of new tissue. While this tissue plays a crucial role in restoring tissue integrity, it lacks the functional properties of the original tissue, resulting in the formation of a scar.

## 2. Factors affecting wound healing

Several factors can influence the wound-healing process, including:

- (1) **Age:** As we age, the overall regenerative capacity of our tissues decreases, leading to delayed wound healing.
- (2) **Nutrition:** Adequate intake of essential nutrients, such as proteins, vitamins, and minerals, is essential for proper wound healing.
- (3) **Chronic conditions:** Chronic conditions like diabetes and autoimmune disorders can impair the body's ability to heal wounds efficiently.
- (4) **Smoking:** Smoking has been shown to significantly delay wound healing by affecting blood flow and impairing immune function.
- (5) **Infection:** Wounds that become infected are more likely to heal slowly and may require additional medical interventions.

Researchers are continually exploring new approaches to enhance wound repair and regeneration. Some recent advancements in this field include:

- (1) **Stem cell therapy:** The use of stem cells has shown promise in promoting tissue regeneration and accelerating wound healing.
- (2) **Growth factors:** Topical application of growth factors, such as platelet-derived growth factor (PDGF), can stimulate cell proliferation and promote wound closure.
- (3) **Smart dressings:** Advanced wound dressings that incorporate technologies like hydrogels, nanofibers, and antimicrobial agents have been developed to provide an optimal environment for wound healing.
- (4) **Tissue engineering:** This emerging field focuses on creating artificial tissues and organs using a combination of cells, biomaterials, and growth factors to promote tissue regeneration.

Wound repair and regeneration are fascinating processes that involve a well-coordinated interplay of cellular and molecular events. Understanding the different phases and mechanisms involved can help in developing effective strategies to promote optimal wound healing. With ongoing advancements in research, the

future holds the promise of even more innovative approaches to enhance wound repair and regeneration <sup>[1,2]</sup>.

When it comes to healing our bodies, nature truly has some incredible tricks up its sleeve. Biomaterials, such as hydrogels, nanofibers, and biodegradable polymers, have revolutionized the field of wound repair. Imagine a material that could not only protect a wound but also provide a suitable environment for healing. That is where biomaterials come in. These specially designed materials have the ability to mimic the extracellular matrix in our bodies, creating the perfect habitat for cells to regenerate and close the wound. From improving the healing process in chronic wounds to creating innovative bandages that can release drugs or promote tissue regeneration, these biomaterials are paving the way for more effective and comfortable wound care <sup>[3,4]</sup>.

Injured soft tissue still needs connective tissue to regenerate. When wound infections are present, the extracellular matrix at the wound site is broken down by the body's own enzymes, including microbial collagenase and elastase, delaying healing and increasing inflammation. Protein-based biomaterial scaffolds serve as a model for skin regeneration by imitating the extracellular matrix at the site of injury. The most prevalent protein in connective tissue, including ligaments, tendons, and cartilage, is collagen. It forms the three-dimensional cellular matrix that gives every tissue its distinct structure, texture, and form <sup>[5]</sup>. Collagen envelops cells. Collagen-based biomaterials have been employed as wound dressings for skin regeneration for the past ten years. Because collagen is a protein that is not needed in heavily infected wounds, wound infections break down collagen and use it as a growing substrate. Collagen biomaterials are stabilized by antimicrobial compounds included in them, which also help eliminate wound bacteria at the site of infection. Antibacterial chemicals are released continuously at the wound surface, resulting in prolonged antibacterial activity that quickly eliminates microorganisms. Utilizing two distinct protein sources to create the scaffold may hasten the healing process of the infected wound's connective tissue. Additionally, one substance can serve as a medication carrier, while another can serve as a scaffold for regeneration <sup>[6,7]</sup>.

One potential approach to regulated drug delivery involves the entrapment of antimicrobial agents within protein-based microspheres that are integrated into a polymer matrix. Gelatin, denatured collagen among protein-based polymers, helps determine the fate of cells and efficiently encapsulates medication. Because of its broad-spectrum activity resulting from the presence of reactive amine and carboxylic acid as functional groups, ciprofloxacin (1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) was selected. It also has good tissue penetration, can treat infections in soft tissues and bones, and exhibits antimicrobial activity against organisms that are resistant to beta-lactam antibiotics. To distribute ciprofloxacin in a sustained release manner, this work addresses the construction and characterization of a reconstituted collagen scaffold impregnated with gelatin microspheres loaded with ciprofloxacin. This would be a likely way to reduce the frequency of normal dressing changes while also managing wound infections effectively and allowing the wound dressing to actively participate in the healing process. This paper reveals the tissue regeneration potential of ciprofloxacin-loaded gelatin microspheres in an albino Wistar rat wound healing model, and the wound healing was assessed via histological investigations.

### **3. Materials and methods**

#### **3.1. Type 1 collagen**

Type 1 collagen was used as the base biomaterial for developing scaffolds for soft tissue repair. The bovine tendon was used as the raw material for the extraction of Type 1 collagen. The extraction process is as follows:

- (1) Obtain bovine tendons from a reliable source, ensuring they are free from any contaminants or diseases.
- (2) Clean the tendons thoroughly to remove any surface debris or blood.
- (3) Cut the tendons into small pieces and remove any excess fat or muscle tissue.

- (4) Place the tendon pieces in a container and cover them with a solution of acetic acid or another suitable acid at a low concentration to break down the tissue and release the collagen.
- (5) Allow the tendon pieces to soak in the acid solution for several hours or overnight at a controlled temperature.
- (6) After soaking, remove the tendon pieces from the acid solution and rinse them thoroughly with distilled water to remove any remaining acid.
- (7) Place the rinsed tendon pieces in a new container and cover them with a solution of an enzymatic proteinase, such as trypsin or pepsin, at a controlled temperature and pH to further break down the tissue and release the collagen.
- (8) Allow the tendon pieces to soak in the enzymatic solution for several hours or overnight at a controlled temperature.
- (9) After the enzymatic treatment, filter the solution to remove any remaining tissue debris and collect the filtrate containing the extracted collagen.
- (10) Concentrate the collagen solution using methods such as ultrafiltration or precipitation to remove impurities and increase the collagen concentration.
- (11) Optionally, further purify the collagen using chromatography or other separation techniques to obtain a highly pure and concentrated collagen extract.
- (12) The extracted collagen can be freeze-dried or otherwise processed to obtain a dry, stable form for storage and use in various applications.
- (13) Characterize the extracted collagen using analytical techniques such as SDS-PAGE, HPLC, and amino acid analysis to confirm its purity and quality.
- (14) Store the extracted collagen in a suitable container at the appropriate temperature and conditions to maintain its stability and functionality <sup>[8]</sup>.

### **3.2. Fabrication of collagen scaffold**

Once the purified collagen is obtained, it can be 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 UV irradiation to improve its stability and mechanical properties.

- (1) **Characterization:** The fabricated scaffold is characterized to assess its physical, mechanical, and biological properties. This may involve testing for porosity, pore size, mechanical strength, and biocompatibility.
- (2) **Sterilization:** 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 involves a series of steps to extract, purify, fabricate, characterize, and sterilize the collagen-based scaffold for use in tissue engineering and regenerative medicine applications <sup>[8]</sup>.

### **3.3. Preparation of gelatin microspheres**

Gelatin microspheres are created using the water-in-oil emulsion method, with 7.5 weight percent ciprofloxacin dissolved in 10 milliliters of the aqueous phase. Using a routine process, 0.5 g of gelatin microspheres loaded with ciprofloxacin were introduced to a specified quantity of collagen solution to incorporate the microspheres into the collagen scaffold <sup>[9]</sup>.

### 3.4. *In vivo* studies

For this investigation, male Wistar albino rats weighing between 150 and 200 g were employed. The animals had unrestricted access to water and were fed commercial pellet food (Hindustan Lever, Bangalore, India). The Institute's ethics committee approved and provided directions for the conduct of the animal experiment. The experimental animals were dressed with designed dressings, a plain collagen scaffold, and a collagen scaffold with an antibiotic after the wound was created <sup>[9]</sup>, while the control group received only gauze dressing. All rats received daily dressing changes, and the antibiotic and designed dressings were changed every two days.

### 3.5. Histological studies

Tissues collected at different intervals were transferred to 10% neutral buffered formalin for 24 hours at 4°C. The formalin-fixed tissues were dehydrated through grades of alcohol, cleared in xylene, and then embedded in paraffin wax (58–60°C melting point). The molds were labeled and stored until use. The deparaffinized sections were stained with hematoxylin and counterstained with eosin <sup>[9,10]</sup>.

## 4. Results and discussion

Porous collagen scaffolds are a type of biomaterial used in tissue engineering for wound repair. These scaffolds are made from collagen, a protein found in the extracellular matrix of connective tissues, and are designed to mimic the natural structure of the body's tissues to support cell growth and tissue regeneration. The porous nature of the collagen scaffold allows for the infiltration of cells and nutrients, providing a suitable environment for tissue regeneration and wound healing. The scaffold also provides mechanical support to the wound site and helps maintain its shape during the healing process. In addition to its structural properties, collagen is also known to have bioactive properties that can promote cell adhesion, migration, and proliferation, as well as modulate the inflammatory response and promote angiogenesis. Porous collagen scaffolds can be used in a variety of wound repair applications, including skin, bone, and cartilage regeneration. They can be designed to degrade over time, allowing for the gradual replacement of the scaffold with new tissue as the wound heals. Overall, porous collagen scaffolds are a promising biomaterial for wound repair, offering a natural and biocompatible environment for tissue regeneration and healing.

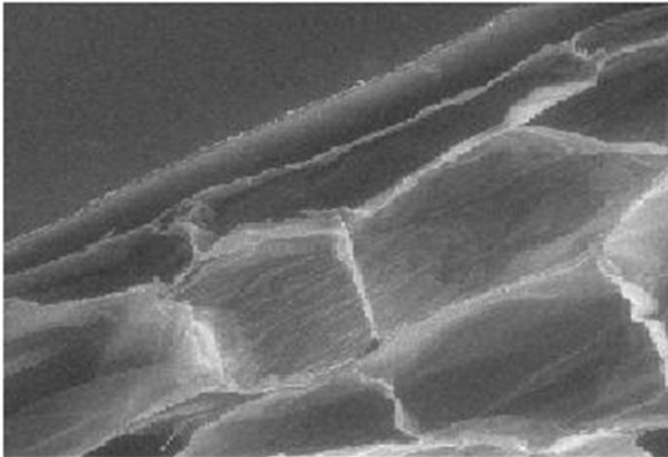
### 4.1. Collagen sponge

The collagen extracted from bovine tendons is used for making the scaffold. The porous scaffold contains pores ranging in size from 500 to 700 µm. The pores in the scaffold help to retain the drug carrier. **Figures 1 and 2** show the porous collagen scaffold prepared via casting.

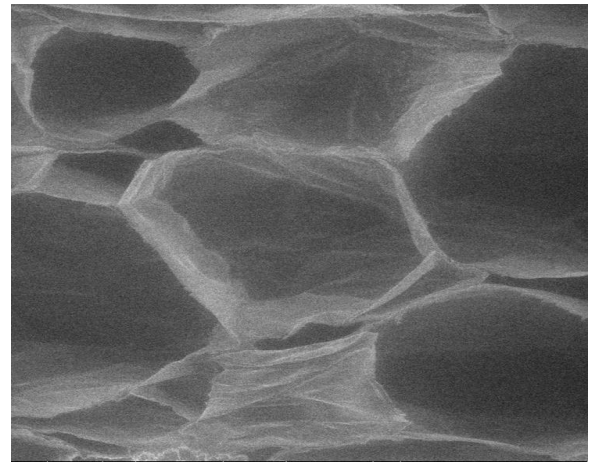
- (1) Cell infiltration: Pores in the collagen scaffold allow for the infiltration of cells, such as fibroblasts and macrophages, which are essential for the regeneration of new tissue.
- (2) Nutrient and oxygen exchange: Pores facilitate the exchange of nutrients and oxygen between the scaffold and the surrounding tissue, promoting the growth and survival of cells within the scaffold.
- (3) Removal of waste products: Pores allow for the removal of waste products and metabolic by-products from the scaffold, maintaining a healthy environment for cell growth and tissue regeneration.
- (4) Angiogenesis: Pores in the scaffold promote the formation of new blood vessels, a process known as angiogenesis, which is essential for delivering oxygen and nutrients to the regenerating tissue.
- (5) Extracellular matrix deposition: Pores provide space for the deposition of extracellular matrix components, such as collagen and elastin, which are essential for the formation of new tissue and the integration of the scaffold with the surrounding tissue.

(6) Mechanical support: Pores in the scaffold help to maintain the structural integrity and mechanical strength of the scaffold, providing support for the regenerating tissue and facilitating the healing process.

Overall, the presence of pores in the collagen scaffold is crucial for promoting cell infiltration, nutrient exchange, waste removal, angiogenesis, extracellular matrix deposition, and mechanical support, all of which are essential for effective wound repair and tissue regeneration <sup>[11,12]</sup>.



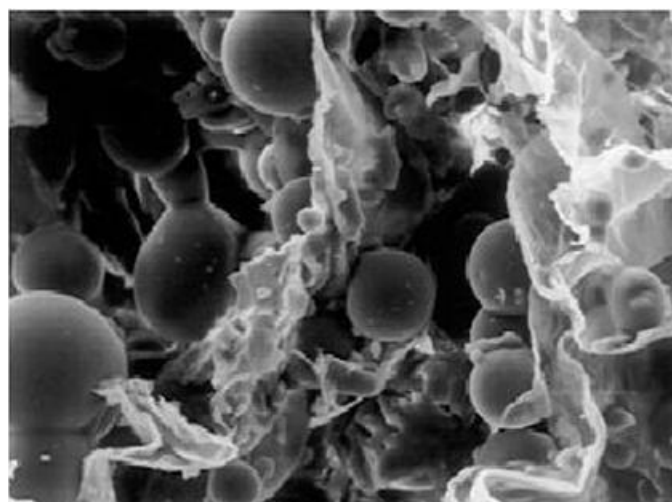
**Figure 1.** Porous collagen scaffold



**Figure 2.** Surface morphology of collagen scaffolds

#### **4.2. Ciprofloxacin-loaded gelatin microspheres impregnated collagen scaffold**

This drug delivery system is designed to release ciprofloxacin, an antibiotic, in a controlled manner. The gelatin microspheres are loaded with ciprofloxacin and embedded within a collagen scaffold, which provides structural support and a suitable environment for tissue regeneration. This system is intended for the treatment of infected wounds or tissue injuries, where the sustained release of ciprofloxacin can help to prevent or eliminate bacterial infections. The gelatin microspheres gradually release the antibiotic, ensuring a steady concentration at the site of application, while the collagen scaffold aids in tissue repair and regeneration. The combination of ciprofloxacin-loaded gelatin microspheres and collagen scaffold (**Figure 3**) offers a promising approach for localized drug delivery and tissue engineering applications, potentially improving the efficacy and safety of antibiotic therapy for various medical conditions.



**Figure 3.** Ciprofloxacin-loaded gelatin microspheres-impregnated collagen scaffold (GMC dressings)

### 4.3. *In vivo* wound healing activity of GMC Dressing

Myofibroblasts, a type of specialized fibroblast present in granulation tissue, are responsible for mediating wound contraction. It is known that the freshly synthesized collagen gel at the healing site contracts these cells. Cell proliferation and granulation tissue synthesis facilitate the revascularization of the wound bed and the regeneration of the extracellular matrix following tissue damage. The proliferative phase of wound healing, which is triggered by the centripetal movement of the surrounding tissues, includes wound contraction as well. Fibroblast activity may be the cause of increased wound contraction in gelatin microspheres-impregnated collagen scaffold (GMC). The presence of bacteria and their metabolites, which influence and inhibit wound contraction and hamper healing, may be the cause of the sluggish rate of wound contraction in rats with an open wound and collagen scaffold. The continuous and gradual release of antibiotics from the dressing may be the cause of the GMC group's higher healing rate. Furthermore, gelatin's presence is crucial for the healing of wounds <sup>[11,12]</sup>.

### 4.4. Histological studies

Histological analysis plays a crucial role in understanding and evaluating wound repair and regeneration processes. By examining tissue samples under a microscope, researchers can gain valuable insights into the cellular and structural changes that occur during healing. This information is essential for developing new therapies and improving clinical outcomes. The typical wound-healing process can be divided into four overlapping phases:

- (1) Hemostasis: This initial phase involves stopping blood loss through platelet aggregation and clotting.
- (2) Inflammation: White blood cells migrate to the wound site to remove debris and fight infection.
- (3) Proliferation: New blood vessels form (angiogenesis), and fibroblasts lay down collagen to create a scaffold for tissue repair.
- (4) Remodeling: The newly formed tissue matures and strengthens, and the wound eventually closes.

Histological analysis can be used to assess each of these phases, looking for:

- (1) In the hemostasis phase: The presence of red blood cells, platelets, and fibrin clots.
- (2) In the inflammation phase: The infiltration of neutrophils, macrophages, and lymphocytes.
- (3) In the proliferation phase: The presence of granulation tissue, composed of new blood vessels, fibroblasts, and collagen.
- (4) In the remodeling phase: The organization and maturation of collagen fibers, as well as the re-epithelialization of the wound surface.

Here are some of the specific features that can be examined in a histological analysis of wound repair and regeneration:

- (1) Cell types: The types and numbers of different cells present in the tissue, such as epithelial cells, fibroblasts, macrophages, and endothelial cells.
- (2) Extracellular matrix: The composition and organization of the extracellular matrix, which provides support and structure to the cells.
- (3) Blood vessels: The presence and density of blood vessels, which are essential for delivering nutrients and oxygen to the healing tissue.
- (4) Nerves: The presence and regeneration of nerves, which are important for restoring sensation to the healed tissue.

Histological analysis is a valuable tool for understanding and evaluating wound repair and regeneration. By providing insights into the cellular and structural changes that occur during healing, it can help researchers

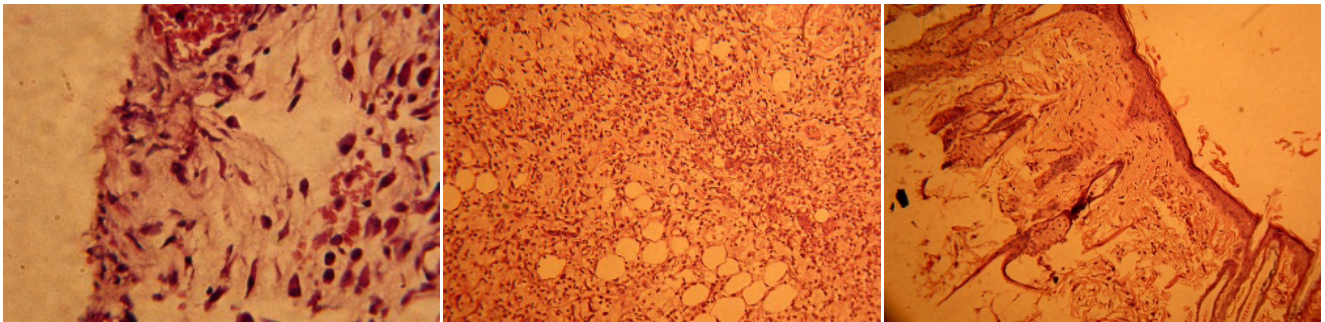
develop new therapies and improve clinical outcomes.

The following interpretation on H&E staining of granulated tissue was carried out to evaluate the wound repair and regeneration by gelatin microspheres impregnated collagen scaffolds.

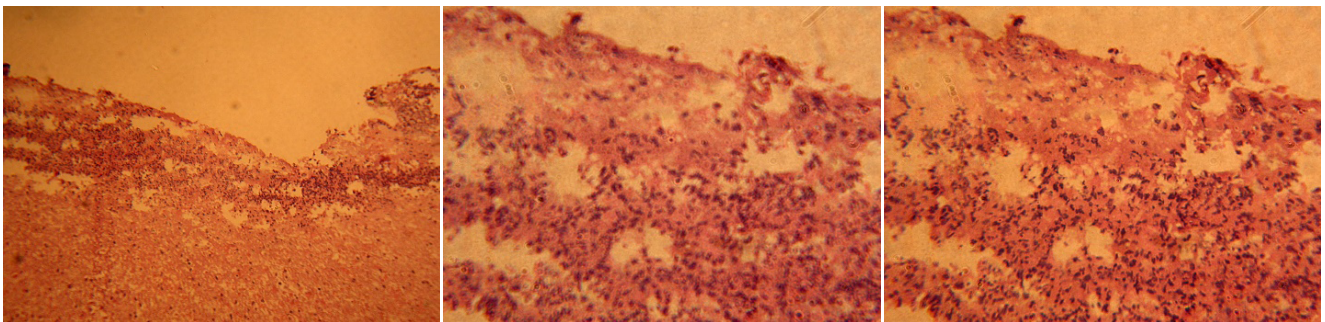
#### 4.4.1. 4th-day investigations

**Figure 3** reveals the wound repair and regeneration on the 4th day. The open wound group confirms that the wound was in the inflammatory stage and contains a lot of neutrophils due to the microbial infection at the wound site. Generally, wound pathogens degrade collagen and other extracellular matrix proteins, resulting in poor regeneration of the dermis and epidermis. In the antimicrobial group (plain ciprofloxacin-incorporated collagen scaffold), there was control of neutrophil synthesis and better formation of the dermis and epidermis on the skin surface. In the case of the GMC, the dermis and epidermis were well regenerated, and the microbial infection was well controlled due to the sustained release of antibiotics from the scaffold. There was no formation of neutrophils, effectively combated by the controlled release of ciprofloxacin from the scaffolds.

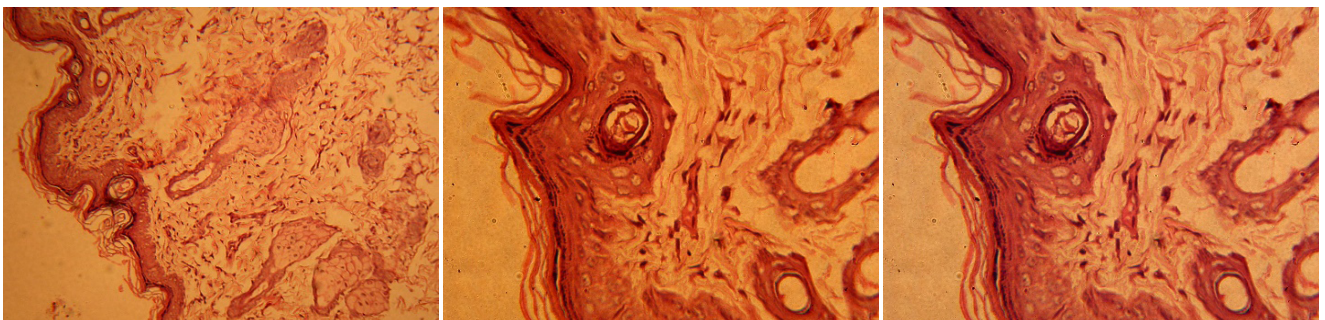
Open wound



Antimicrobial-incorporated collagen scaffold



Ciprofloxacin-loaded GMC



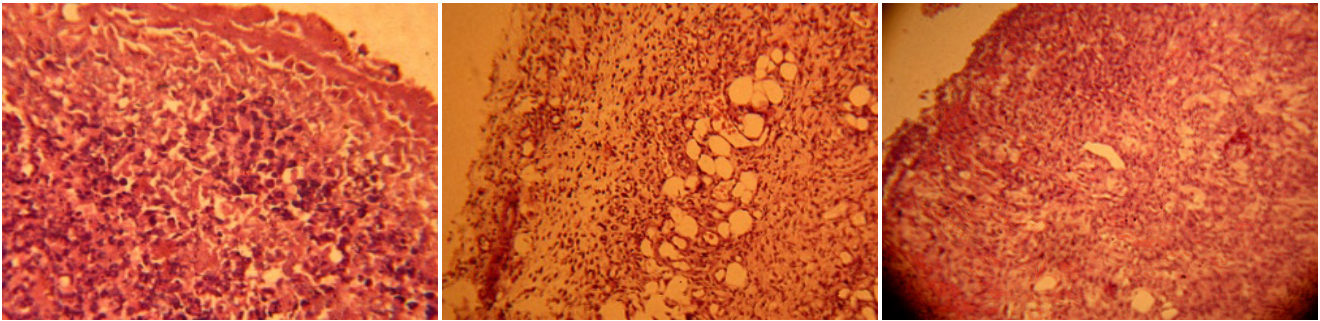
**Figure 3.** H&E staining of granulated tissue taken from the 4th day



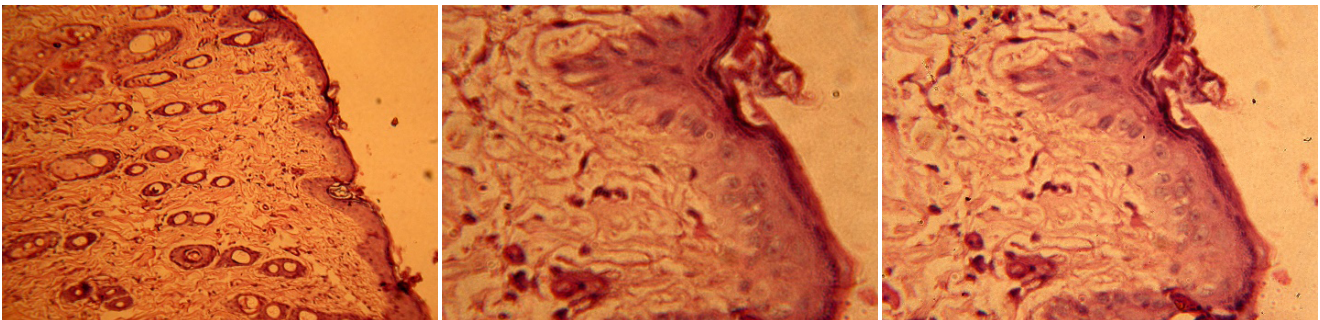
#### 4.4.2. 8th-day investigations

**Figure 4** shows the wound regeneration stage of the granulated tissue collected on the 8th day (animal groups). On the 8th day, the granulated tissue shows a lack of dermis and epidermis on the wound surface and contains neutrophils. In the category of antimicrobial incorporated collagen scaffold, good formation of the dermis and epidermis has been observed on the wound surface. In the case of GMC, the dermis and epidermis were well regenerated, and wound repair and regeneration were accelerated by combating the wound pathogens with ciprofloxacin released from the scaffolds.

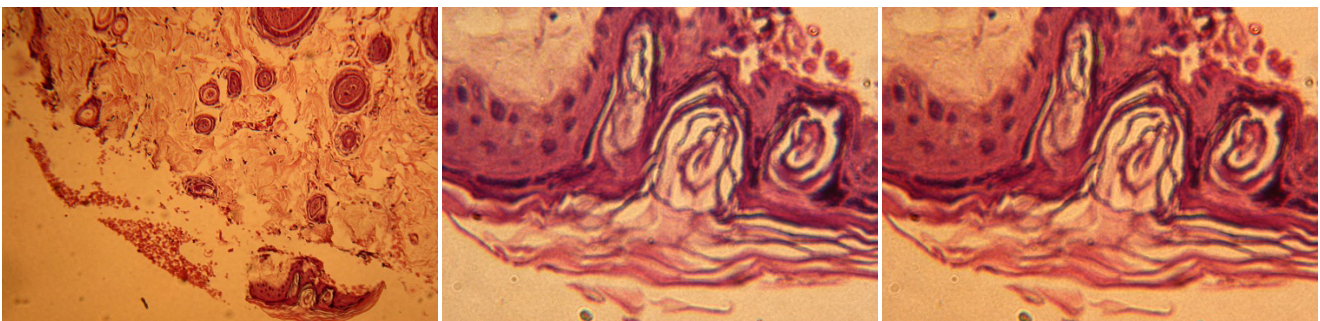
Open wound



Antimicrobial-incorporated collagen scaffold



Ciprofloxacin-loaded GMC

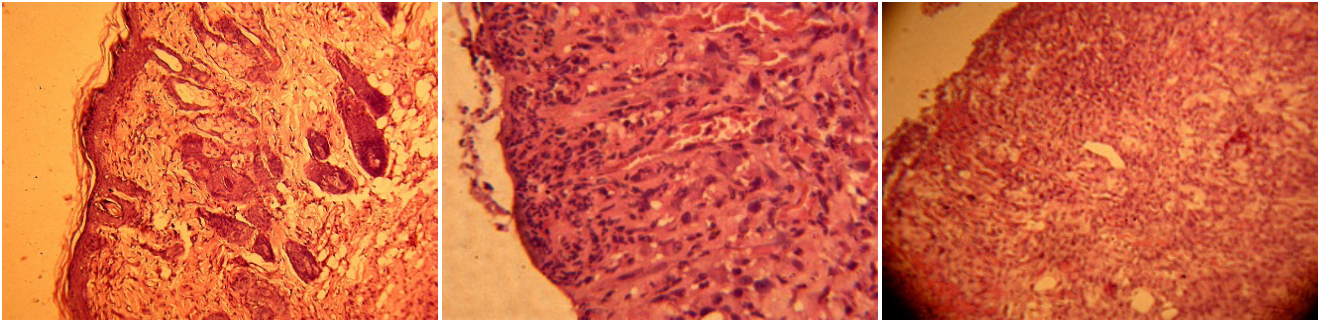


**Figure 4.** H&E staining of granulated tissue taken from the 8th day

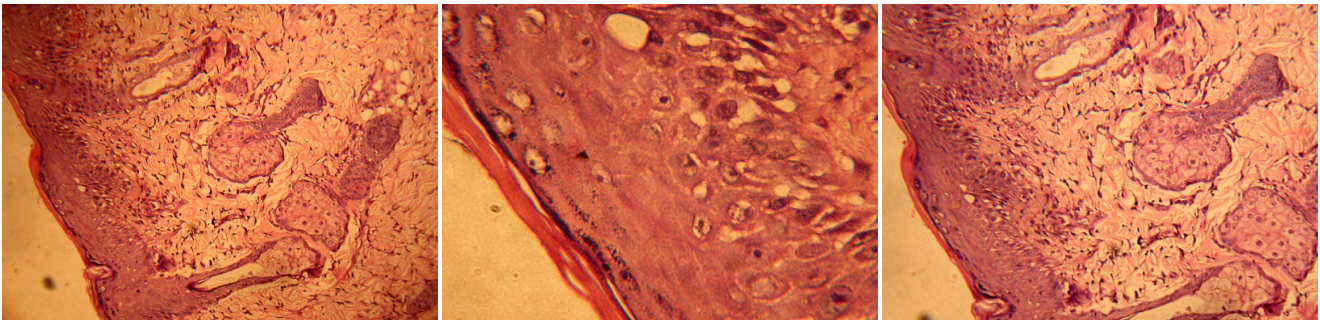
#### 4.4.3. 12th-day investigations

**Figure 5** shows the wound healing stages on the 12th day by assessing granulated tissue through H&E staining. The open wound group shows poor formation of the dermis and epidermis on the wound surface due to microbial pathogens. Other groups show that the dermis and epidermis were well-formed on the wound surface. In addition, well-defined dermis and epidermis were regenerated in the case of GMC dressings.

Open wound



Antimicrobial-incorporated collagen scaffold



Ciprofloxacin-loaded GMC

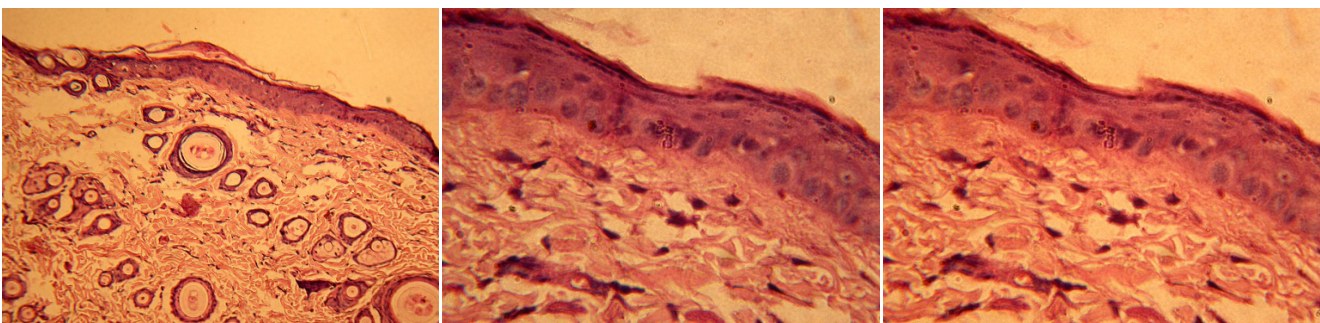
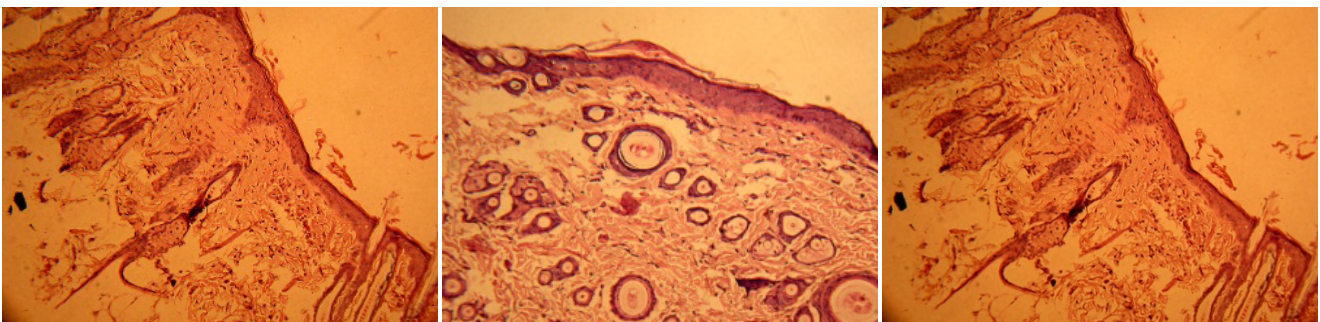


Figure 5. H&E staining of granulated tissue taken from the 12th day

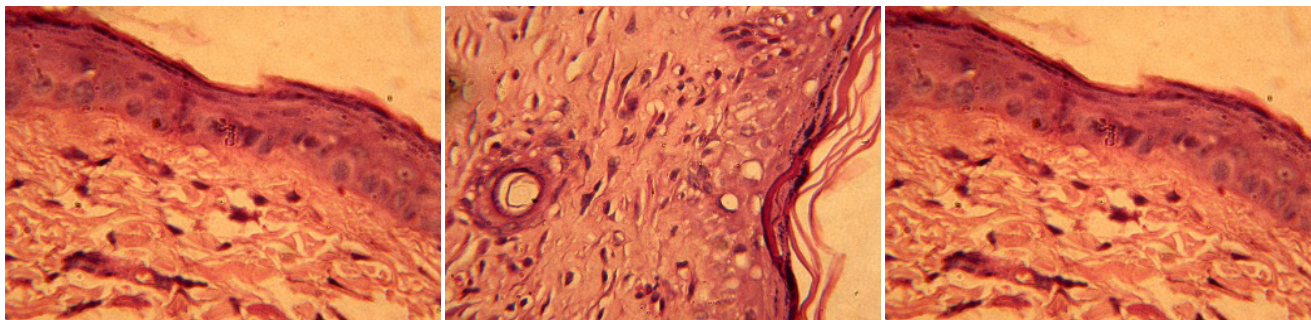
#### 4.4.4. 16th-day investigations

Figure 6 confirms the final stage of wound repair and regeneration via collagen scaffolds. It confirms that the granulated tissue of the open wound group did not effectively regenerate the wound and still contained neutrophils. The dermis and epidermis were poorly regenerated. Other scaffolds show the effective regeneration of the wound.

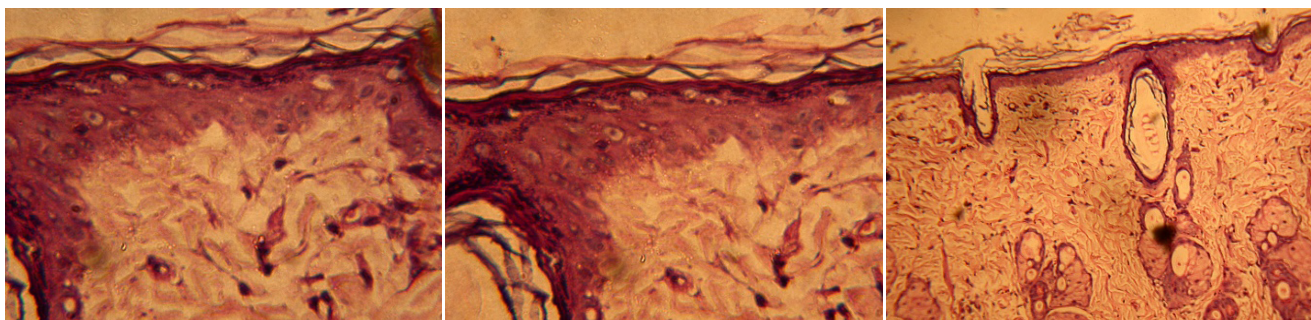
Open wound



#### Antimicrobial-incorporated collagen scaffold



#### Ciprofloxacin-loaded GMC



**Figure 6.** H&E staining of granulated tissue taken from the 16th day

The skin is the body's largest organ system, functioning as a protective, regulatory, sensory organ and a mechanism for immunological monitoring. Its primary purpose is to act as an environmental barrier. The wound healing process is automatically triggered by tissue damage, resulting in cell death, the breakdown of extracellular connective tissue components, and loss of blood vessel integrity. Generally, wound infections impede the healing process, while wound pathogens prevent collagen formation at the wound site. The pathogenicity, virulence, and immunocompetence of the microorganism, as well as the host's immune system, all influence the development of a wound infection.

Extracellular infections are more prevalent in wounds than intracellular infections, with many pathogens relying on extracellular enzyme synthesis to penetrate deep into the tissue. Microbial colonies form biofilms, which are wrapped in slime and adhere to surfaces, providing defense against antimicrobial agents, antibiotics, and phagocytosis. Chronic wound biofilms may be related to the inability to heal. *Pseudomonas aeruginosa*, *Streptococcus* species anaerobes, and *Staphylococcus aureus* are the main bacteria linked to wound infections. Collagenase is one of the extracellular enzymes secreted by *Pseudomonas aeruginosa* [11].

Collagenase does not damage collagen found in healthy tissue; instead, it hydrolyzes collagen in necrotic tissue. Topical application of antimicrobial or wound-healing agents in the form of gel or solution to the wound site will quickly disperse and be removed, making it difficult to maintain an effective local concentration, which will cause the wound to heal more slowly. Both microbial infections and the quantity of medication required for the same therapeutic benefit can be reduced using controlled-release formulations. Gelatin microspheres serve as both a medication carrier and collagen scaffold support for skin regeneration in current drug delivery systems. Collagen has proven to be extremely appealing for mending wounds, as wound fluid may be absorbed in enormous volumes by collagen scaffolds. These scaffolds are porous enough to allow for medication absorption and delivery. A drug delivery system that stops further bacterial growth and administers an adequate amount of medication at the site of action is desirable. Before the release rate stabilizes, a sizable initial drug bolus is released in many controlled release formulations as soon as the drug is placed in the release medium,

usually called “burst release.”

The inhibition of bacterial DNA gyrase and topoisomerase IV enzymes by fluoroquinolones results in their bactericidal effect. Topoisomerase IV is involved in the partitioning of chromosomal DNA during cell wall division, while DNA gyrase is necessary for the transcription, replication, and repair of bacterial DNA. Fluoroquinolones stop bacterial reproduction by blocking these enzymes, maintaining bacterial DNA in a supercoiled form within the cell.

It has been demonstrated that Type 1 collagen derived from bovine tendons is tissue biocompatible, mildly antigenic, and appropriate for creating scaffolds for tissue engineering applications [8-10]. The created porous collagen scaffold promotes cell fate processes and increases the biomaterial’s surface area, enhancing the interaction between cells and the matrix. Biomaterials based on collagen have been employed for soft tissue regeneration in the last ten years. Collagen biomaterials must be used with antimicrobial treatments for infected cutaneous wounds [13]. Because collagen is a protein, wound infections can build biofilms at the wound site by using collagen as a growth substrate. As a medication carrier, gelatin microspheres are employed. In addition to its ability to entrap drugs, gelatin supports tissue regeneration. Collagen and gelatin scaffolding together may promote improved skin regeneration at the site of damage. SEM was used to further examine the dimensions and form of the gelatin microspheres loaded with ciprofloxacin. Gelatin microspheres that have been manufactured range in size from 1000 nm to 10 µm. The prepared microspheres were compact and spherical, as seen in **Figure 2**. In water, the particles were more easily diffused and less aggregating. The yield of drug-loaded gelatin microspheres produced by the water-oil emulsion approach is 78.6% drug encapsulated in microspheres and 88.2% during preparation and recovery. The surface architecture of the collagen scaffold impregnated with microspheres is seen in **Figure 1**. The holes in the collagen scaffold allow the physical entrapment of gelatin microspheres. The simple porous collagen scaffold’s pore size varies between 500 and 600 µm, according to the SEM observation [7]. Gelatin microspheres are efficiently physically encapsulated by the scaffold’s pore size. The microspheres are evenly dispersed inside the collagen scaffolds’ pores, as seen by the SEM observation.

A drug delivery system that stops further bacterial growth and administers an adequate amount of medication at the site of action is desirable. The medicine produced from the sponge exhibits a distinct zone of inhibition that regulates the development of the two cultures that were injected. The collagen scaffold’s large pores effectively absorb and administer drugs, and the microspheres made of gelatin absorb water and keep the wound’s surface moist.

Myofibroblasts, a kind of specialized fibroblast present in granulation tissue, are responsible for mediating wound contraction. It is known that the freshly synthesized collagen gel at the healing site contracts these cells. Cell proliferation and granulation tissue synthesis facilitate the revascularization of the wound bed and the regeneration of the extracellular matrix following tissue damage. The proliferative phase of wound healing, which is triggered by the centripetal movement of the surrounding tissues, includes wound contraction as well. Because of the increased fibroblast activity, **Figure 4** illustrates higher wound contraction in the GMC group. The presence of bacteria and their metabolites, which influence and inhibit wound contraction and hamper healing, may be the cause of the sluggish rate of wound contraction in rats with an open wound and collagen scaffold. The continuous and gradual release of antibiotics from the dressing may be the cause of the GMC group’s higher healing rate. Additionally, gelatin’s presence is crucial for skin regeneration.

## 5. Conclusion

This work addresses the construction and characterization of a reconstituted collagen scaffold impregnated with gelatin microspheres loaded with ciprofloxacin to distribute ciprofloxacin in a sustained release manner. This

method can potentially reduce the frequency of normal dressing changes, manage wound infections effectively, and allow the wound dressing to actively participate in the healing process. Histological analysis of granulated tissue confirms that ciprofloxacin-loaded microsphere-impregnated collagen scaffolds regenerate the dermis and epidermis more effectively than ciprofloxacin-incorporated collagen scaffolds. Given this correspondence, the microsphere-incorporated collagen scaffolds would be an effective wound dressing for infected dermal wounds, providing sustained release of antibiotics into the wound surface.

## Disclosure statement

The author declares no conflict of interest.

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