Bone and Arthrosurgery Science, 2024, Volume 2, Issue 4
http://ojs.bbwpublisher.com/index.php/BAS
Online ISSN: 2981-8222

A Review on Dermal Wound Repair and Regeneration Using Antimicrobial-Loaded Microspheres Incorporated into Bovine Collagen Scaffold

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Abstract: In this paper, the developed microsphere-incorporated collagen scaffold presents a promising solution for delivering drugs over wound surfaces in a uniform and sustained manner. The biomaterials utilized in this system are of natural origin, minimizing potential toxicity from core materials, and collagen and gelatin exhibit active wound-healing properties. The collagen scaffold used for the delivery device offers several advantages, including minimizing dressing frequency, facilitating easier examination, and providing added aesthetic value. Furthermore, the protein-based biomaterial scaffold acts as a template for the extracellular matrix in the skin, enhancing the maturation of wounds and enabling the transition to the accelerated production of mature and aligned collagen fibers. The controlled release of antimicrobial drugs through denatured collagens and gelatin microspheres, as well as the efficient healing observed in *in vivo* studies, demonstrate the potential of this system for effective wound care. Overall, the incorporation of ciprofloxacin-loaded microspheres in porous collagen scaffold offers sustained releases of ciprofloxacin in infected environments, as supported by histological examination showing well-formed epidermis and dermis in the connective tissue of the skin. The system's ability to heal full-thickness wounds within a significantly shorter time frame compared to antibiotic-incorporated collagen sponges further emphasizes its efficacy in wound healing.

Keywords: Gelatin microspheres; Collagen; Controlled release; Drug encapsulation; Drug entrapment; Scanning electron microscopy; *In vivo* studies; Wound healing; Wound infections; Ciprofloxacin

Online publication: September 9, 2024

1. Introduction

Holistic dermal wound repair and regeneration is a highly intricate process that involves a myriad of cellular and molecular mechanisms. Upon injury, the body's first response is initiating several events that are aimed at wound healing and closure of the torn skin & skin integrity. Hemostasis (clot formation), inflammation, proliferation (cell growth), and remodeling (tissue reconstruction) can be interpreted as overlapping phases of

the process. Hemostasis is when blood vessels contract and platelets—native cells of the body—give rise to a clot. In the inflammation stage, the immune cells scavenging for debris remove dead tissue and pathogens. Consequently, fibroblasts and keratinocytes multiply and migrate to the wound site to bring about new tissue. The final phase of the process is when the initial scar tissue undergoes the remodeling phase and as a result, the stronger and better-organized collagen fibers are found. Various factors can affect the speed and success of dermal wound healing, including age, overall health, and nutrition as well as wound size and depth. Such phases of the repair process occur when healing is delayed or progresses at a slower pace. In these cases, complications such as chronic wounds or excessive scar tissue formation may arise. Researchers have found new techniques to enhance dermal wound healing and regeneration, including stem cells, growth factors, and biomaterials, along with advanced tissue engineering techniques. Understanding the complex processes the body undergoes during healing is a key focus for doctors, as they aim to develop supplemental drugs that accelerate skin repair [1,2].

Dermal wounds are the most common type of injury, often leading to severe infections due to contamination from farm environments. Agricultural areas are rich in bacteria and pathogens that can easily infect wounds and form biofilms, resulting in a decrease in the workforce and hindering daily farm operations. Currently, the emergence of drug-resistant pathogens is another common issue in the treatment of an infected wound. As a consequence, wound closure becomes a challenging task and requires a novel treatment for better wound healing. The regeneration of connective tissue in the injured soft tissue is still exigent. The presence of pathogens at the wound site delays healing, produces inflammation, and causes degradation of an extracellular matrix at the wound site by its native enzymes such as microbial collagenase and elastase. The scaffolds made from protein-based biomaterials act as a template for dermal regeneration and mimic the extracellular matrix at the injured site. Collagen is the most abundant protein in connective tissue such as cartilage, tendon, and ligament; it surrounds the cells and forms the three-dimensional cellular matrix of all tissues, giving each its characteristic structure, texture, and shape [3,4].

While collagen is a popular and effective biomaterial for wound healing, it has certain disadvantages when used in the treatment of infected dermal wounds. These limitations may affect its efficacy and suitability in certain clinical scenarios. The disadvantages of using collagen biomaterial for infected dermal wounds are as follows:

(1) Susceptibility to infection: Collagen is naturally biodegradable, which is beneficial in non-infected wounds. However, in infected wounds, collagen can serve as a substrate for bacterial growth, potentially exacerbating the infection. Bacteria can colonize the collagen material, making it difficult to control infections without the use of additional antimicrobial agents.

(2) Limited antimicrobial properties: Collagen lacks inherent antimicrobial properties, meaning it does not directly combat infections. In infected wounds, collagen alone may be insufficient to prevent or reduce bacterial load, requiring the use of supplemental antimicrobials, such as silver, antibiotics, or other antimicrobial agents, to manage infection.

(3) Degradation in the presence of infection: Infected wound environments often contain elevated levels of proteases and other enzymes that can accelerate the degradation of collagen. This rapid breakdown of collagen can lead to premature loss of its structural integrity, reducing its effectiveness in providing a scaffold for tissue regeneration.

(4) Potential for immunogenicity: Depending on the source of the collagen (bovine, porcine, marine, etc.), there is a risk of an immune response, particularly in the presence of infection. Infected wounds have led to an elevated immune response, and the introduction of collagen from animal sources could trigger further inflammation or adverse reactions in some patients.

(5) Suboptimal mechanical properties in infected environments: Infected wounds often present with excess exudate and inflammatory factors, which can compromise the mechanical stability of collagen-based materials. Collagen may lose its tensile strength and degrade too quickly in such environments, reducing its ability to support wound healing effectively.

(6) Risk of biofilm formation: Collagen materials may become a site for biofilm formation, where bacteria adhere and form protective colonies that are resistant to antibiotics and immune system attacks. This can make treating infections in the wound more difficult, prolong healing, and lead to chronic wound conditions.

(7) Limited effectiveness in severe or chronic infections: In cases of severe or chronic infections, collagen-based treatments may be less effective because they do not directly address the underlying infection. In such cases, the primary focus should be on controlling the infection, before collagen can effectively promote wound healing.

(8) Higher costs and limited availability: Collagen-based products, especially those derived from specific sources (e.g., recombinant or marine collagen), can be expensive and may not be readily available in all healthcare settings. This may limit their widespread use, particularly in resource-constrained environments, where cost-effective alternatives may be preferred.

To overcome some of these disadvantages, collagen biomaterials are often combined with antimicrobial agents (e.g., silver or antibiotics) or cross-linked to improve their resistance to degradation and enhance their stability in infected wound environments. These combined products aim to provide the benefits of collagen in wound healing while addressing the challenges posed by infection. While collagen is an excellent biomaterial for promoting wound healing, its effectiveness in infected dermal wounds can be limited due to its susceptibility to bacterial colonization, lack of antimicrobial properties, and potential for rapid degradation. Additional strategies, such as combining collagen with antimicrobial agents or enhancing its mechanical properties, are often necessary to improve its performance in treating infected wounds [3].

2. Infected dermal wound healing

Primary factors for infecting open dermal wounds include immunocompetent host, pathogenicity, and virulence of microorganisms. However, several factors contribute to microbial pathogenicity, which is affected by the genetic and environmental impact of pathogens. Structural features, enzymes, and metabolites produced by wound pathogens at the site of open wounds further increase the virulence and pathogenicity of the organism. The possession of capsules, such as in *Pseudomonas aeruginosa*, protects bacteria from phagocyte-mediated killing and complement activation, enhancing their ability to evade the host immune system. Fine surface appendages (pili) that extend from many bacteria (e.g. *Pseudomonas aeruginosa* and *Escherichia coli*) allow attachment to target host cells, which is often the first step in the infection process. In *Staphylococcus* and *Streptococcus* infections, polysaccharide components of the bacterial cell walls facilitate adherence to extracellular matrix components in target tissue like fibronectin or collagen. In open wounds, extracellular infection is more common than intracellular infection and many pathogens rely on the production of extracellular enzymes, which help to invade the tissue and degrade the extracellular proteins such as collagen and elastin in the extracellular matrix. Microbial biofilms get attached to surfaces forming a slime layer providing protection against phagocytosis, antibiotics, and antimicrobial agents. The formation of biofilms in chronic dermal wounds may be linked to the failure of wound healing [5-8]. As a consequence, targeting drugs to infected dermal wounds, as it requires eradicating wound pathogens and preventing biofilm formation at

the wound site. The acquisition of microbial species in wounds can lead to three clearly defined outcomes, as shown in **Figure 1**.

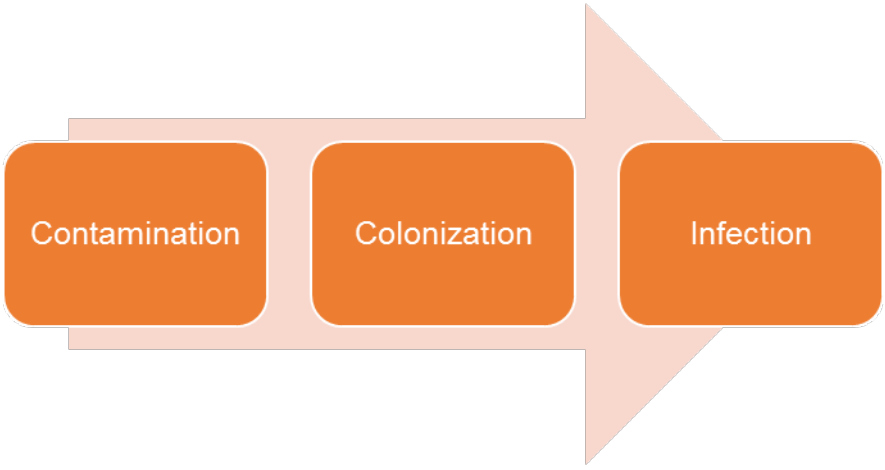


Figure 1. Steps in the infected wound site

2.1. Pathogens causing wound infections

The main pathogens causing wound infections include *Staphylococcus aureus*, *Streptococcus* species, anaerobes, and *Pseudomonas aeruginosa*. These pathogens secrete enzymes like collagenase and elastase, which degrade tissue components, inhibit fibroblast growth, and delay wound healing. They produce tissue-damaging exoenzymes, making them significant contributors to infection. Understanding their mechanisms can aid in developing effective treatment strategies for acute and chronic wounds [9-12].

2.2. Consequences of infected dermal wound

Staphylococcus aureus, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa* are considered the most common wound pathogens with $\geq 10^3$ CFU/ g of tissue for wound infection. As a result, infected dermal wounds experience delayed regeneration of both the dermis and epidermis. This delay is largely due to the degradation of the extracellular matrix caused by enzymes secreted by wound pathogens. Bacterial enzymes with collagen-degrading abilities have been known for centuries, although their precise nature and substrate specificity remain controversial. Collagenolytic enzymes were isolated from cultured filtrates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* and then evaluated for their mode of action against degradation of extracellular matrix in the dermal wound [13-17].

3. Collagen as a biomaterial for wound repair

Collagen is one of the most widely used biomaterials for wound repair due to its unique biological properties that support tissue regeneration and healing. It is the primary structural protein in the extracellular matrix of various tissues, including skin, tendons, cartilage, and bones. It provides mechanical strength, structure, and support to tissues and plays a crucial role in the wound-healing process. There are several types of collagen, but type I is the most abundant and commonly used in wound healing applications. Collagen's biocompatibility, biodegradability, and ability to promote cell adhesion, migration, and proliferation make it an ideal biomaterial for wound healing. It mimics the body's natural extracellular matrix, providing a scaffold that supports the migration of cells involved in the healing process, such as fibroblasts and keratinocytes. Collagen also helps in the formation of new blood vessels (angiogenesis) and facilitates the deposition of new collagen by cells,

leading to tissue regeneration [18,19]. Below are some characteristics and advantages of the application of collagen in wound healing:

(1) Hemostasis and inflammation: Collagen can absorb exudates and provide a moist wound environment, essential for controlling bleeding and reducing inflammation.

(2) Proliferation phase: Collagen scaffolds support the proliferation of fibroblasts and the production of new extracellular matrix, essential for tissue repair. It also promotes re-epithelialization by supporting the migration of epithelial cells.

(3) Remodeling phase: Collagen scaffolds degrade over time, allowing newly formed tissue to remodel and mature, eventually forming a healed wound with restored tissue function.

(4) Collagen dressings: Collagen dressings are applied directly to wounds to create a moist environment conducive to healing. They are used for chronic wounds, pressure ulcers, diabetic ulcers, and burns. These dressings may also contain additional agents such as silver or growth factors to further promote healing.

(5) Collagen scaffolds: Collagen scaffolds provide a three-dimensional framework for cell attachment and tissue regeneration. They are particularly useful in treating large wounds or wounds that require structural support, such as deep burns or surgical wounds.

(6) Collagen gels and matrices: Collagen can be used as injectable gels or matrices to fill wound spaces, support tissue growth, and enhance healing in a minimally invasive way.

(7) Collagen composites: Collagen is often combined with other biomaterials, such as elastin, hyaluronic acid, or synthetic polymers, to enhance its properties and provide customized solutions for different types of wounds.

(8) Biocompatibility: Collagen is naturally recognized by the body, minimizing the risk of rejection or adverse reactions.

(9) Bioactivity: Collagen promotes cellular activities such as adhesion, migration, and proliferation, all critical for tissue repair.

(10) Degradability: Collagen degrades naturally in the body, meaning there is no need for removal, and it allows for gradual tissue replacement during the healing process.

(11) Moisture balance: Collagen dressings help maintain a moist wound environment, which is crucial for effective wound healing.

While collagen is highly effective for wound repair, there are some challenges, such as variability in collagen sources (bovine, porcine, or marine), potential immunogenicity, and rapid degradation in certain applications. To address these, researchers are exploring cross-linking techniques to improve the mechanical strength and longevity of collagen-based materials, as well as the development of synthetic collagen-like proteins.

Collagen remains a vital biomaterial in wound repair due to its natural role in tissue structure and regeneration. Its ability to promote healing, support new tissue formation, and provide a scaffold for cell attachment makes it a go-to material for clinicians and researchers. Ongoing advancements in collagen-based products continue to enhance their effectiveness and broaden their applications in wound management [20].

Collagen is the most prominent protein in the extracellular matrix and the scaffold made from type 1 collagen mimics the extracellular matrix behavior leading to an effective tissue regeneration process. The extracellular matrix-based scaffolds are used to trigger cell proliferation, differentiation, and migration into the repair/restoration/regeneration process. The collagen scaffold is responsible for tissue scaffolding, tensile strength, cell-extracellular matrix interactions, cell-cell interactions, and fibroblast activation in wound repair.

In the past decade, collagen-based biomaterials have been used as wound dressings for dermal regeneration. Numerous forms of reconstituted collagen biomaterials are in commercial application [21,22]. These products are summarized in **Table 1**.

Table 1. Products made from collagen

Collagen form	Product	Company
Partially purified skin	Gelfoam	Pfizer
Collagen sponge	Helistat	Integra LifeSciences
	Instat	Johnson & Johnson
	ActiFoam	MedChem
	SkinTemp	BioCor
Collagen fibre	Helitene	Integra LifeSciences
	Instat Fibrillar	Johnson & Johnson
	Avitene	MedChem
Collagen powder	BioCore	Medifil
Collagen composite dressing	Fibracol	Johnson & Johnson
	Biobrane	UDL Laboratories
Hydrolyzed collagen	Chronicure	Derma Sciences

Generally, collagen is resistant to proteolysis. However, collagen could be degraded by matrix metalloproteinases through cleaving of single-strand regions of the collagens. The high biocompatibility and intrinsic biodegradability of collagen by endogenous collagenases make exogenous collagen ideal for use in biomedical and tissue engineering applications. The collagen-based materials could be classified into decellularized collagen matrices that maintain the original tissue properties and extracellular matrix structure; and more refined and reconstituted scaffolds prepared via extraction, purification, and collagen polymerization.

The collagen sponge/scaffold, made of type 1 collagen, is a versatile biomaterial used in various biomedical applications. These sponges are created by lyophilizing collagen solutions and the porosity is controlled by adjusting collagen content and freezing rate. They are commonly used for soft tissue augmentation and dermal wound healing, absorbing exudate, maintaining a moist environment, and protecting against infection. When combined with other proteins, collagen sponges create composites with enhanced properties. They can also serve as a scaffold for studying wound healing patterns and promoting cell motility and regeneration. Collagen sponges are particularly effective in dermal wound healing and can be used for delivering growth factors and antimicrobial agents. They promote the formation of collagen fibers and tensile strength in wounds. The scaffolds can be chemically modified to control drug delivery and immobilize bioactive molecules. When cells interact with collagen scaffolds, collagen synthesis increases, leading to tissue regeneration. The sponges are eventually degraded and replaced by native collagen based on the degree of cross-linking. Incorporating mesenchymal stem cells into collagen scaffolds could offer a promising approach to wound treatment and tissue engineering. Overall, collagen-based sponges present a promising platform for advanced wound care and regenerative medicine applications [23-26].

4. Collagen biomaterial for infected dermal wound

Collagen, a natural protein, can be utilized in biomaterials to combat infections in wounds. By incorporating antimicrobial agents into collagen biomaterials, wound pathogens can be targeted and eradicated effectively. Combining different protein materials in scaffolds can accelerate connective tissue regeneration in infected wounds. Gelatin, denatured collagen, is highlighted for its ability to encapsulate drugs and support cell fate

processes. Ciprofloxacin is recommended for its broad-spectrum antimicrobial activity and tissue penetration, making it effective against various infections (**Figure 2**). Controlled drug delivery methods, such as using protein-based microspheres, can further enhance treatment outcomes.

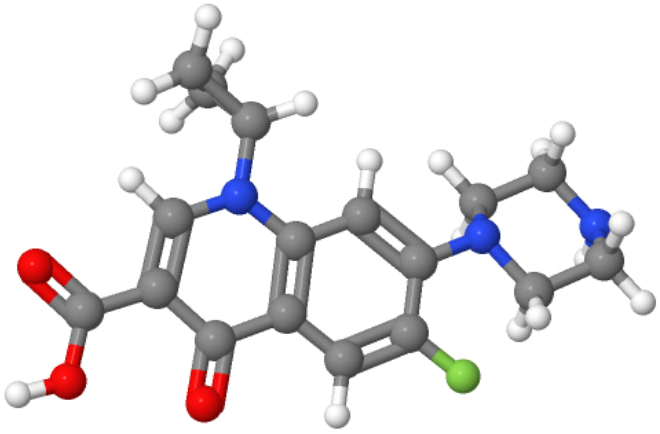


Figure 2. Structure of ciprofloxacin

Ciprofloxacin is used to treat numerous bacterial infections including bone and joint infections, abdominal infections, respiratory infections, skin infections urinary tract infections, among others. The mechanism of antimicrobial action of ciprofloxacin is the inhibition of DNA gyrase and topoisomerase type II and IV enzymes in the wound pathogens, resulting in the inhibition of cell wall synthesis, protein synthesis, nucleic acid replication and transcription, and synthesis of essential metabolites. In infected wounds, methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* are the most common pathogens associated with soft tissue infection. Open wounds can be contaminated with beta-hemolytic streptococci and *Enterobacteriaceae*. These pathogens are susceptible to the antimicrobial action of ciprofloxacin. Thus, ciprofloxacin is suitable as a model drug for incorporation in the collagen scaffold and loading into the gelatin microspheres for controlled delivery into the wound.

Numerous scaffolds have been used for skin regeneration. The advantages and disadvantages of different scaffolds used in skin regeneration are summarized in **Table 2**. Microencapsulation of the antimicrobial agents overcomes a number of shortcomings in drug delivery such as low solubility, high potency, and/or poor stability. The microspheres-based controlled delivery system gives a prolonged release of the drugs to the site of injury or infection. In infected dermal wound healing, the pathogen biofilm degrades the collagen scaffold, effectively utilizing the scaffold as a nutrient source for microbial growth. This degradation process delays wound regeneration and exacerbates inflammation. The efficiency of microencapsulation of the drugs depends on the type of polymer, the polymer molecular weight, the copolymer composition, the nature of any excipients added to the microsphere formulation (e.g., for stabilization of the therapeutics), and the microsphere size. The rate of controlled release of drugs from the microspheres depends on the degradation rate of the polymer.

Gelatin is chosen as a biodegradable polymer for microencapsulation of antimicrobials in developing collagen scaffolds for infected tissue repair. It is a hydrolyzed form of type I collagen that is used widely in the pharmaceutical and medical industries, including tissue engineering applications and regenerative medicine.

Gelatin contains binding sites for cell attachment and cell fate process. It can be broken down by cellular action through the secretion of specific enzymes known as matrix metalloproteinases. This enzymatic hydrolysis of gelatin does not produce any toxic by-products and is completely degraded by cellular processes. Gelatin offers potential solubility in aqueous solutions at physiological pH, making it a biocompatible choice for drug

delivery. In contrast, microencapsulation of drugs using synthetic polymers often requires organic solvents, which can negatively impact the bioactivity of the drugs and pose toxicity risks to cells.

In infected wounds, the microbial enzymes from wound pathogens can degrade gelatin and collagen. The microbial degradation and dissolution of drug-loaded gelatin microspheres offer controlled release.

Microsphere-incorporated scaffolds have reaped outstanding attention in tissue engineering and regenerative medicine. In tissue engineering scaffold design, the incorporation of microspheres into the scaffold is mainly used for the controlled or sustained release of drugs or other bioactive molecules into the site of injury. The nano/microspheres in scaffold design have promising regeneration of tissue and are capable of tailoring the controlled release by adjusting the molecular weight of polymers. For example, a rapid release of the drug from the scaffold can be achieved by lowering the molecular weight of the polymer. The recent approach to microsphere scaffold provides easy fabrication, controlled morphology, and physicochemical properties, resulting in well-tailored pharmacokinetics of the encapsulated molecules. Recently, nano/microspheres such as poly(lactic-co-glycolic acid) (PLGA), and natural polymers such as collagen or gelatin microspheres were developed for dermal regeneration scaffolds for the controlled release of drugs such as antibiotics or growth factors. The size of microspheres and type of polymers used in microencapsulation in the scaffolds can be tailored for the controlled release of proteins or drugs. Currently, gelatin-based microsphere scaffolds are used as microcarriers for stem cells for skin regeneration. This investigation confirms the utility of microsphere scaffolds for effective skin tissue regeneration. The bioactive scaffolds and their applications are summarized in **Tables 2** and **3**.

Ciprofloxacin-loaded gelatin microspheres are a promising drug delivery system due to their ability to control drug release and improve therapeutic efficacy. Gelatin, a biocompatible and biodegradable material, serves as an ideal matrix for encapsulating drugs like ciprofloxacin. The process of preparing gelatin microspheres involves techniques such as emulsification, coacervation, or crosslinking, which allow for the formation of uniform-sized particles that can release the drug in a controlled manner. The size and composition of the microspheres can be tailored to achieve specific drug release profiles and target different sites in the body. Once administered, the gelatin microspheres protect the drug from degradation and enhance its absorption, leading to prolonged circulation time and improved bioavailability. This delivery system also reduces the frequency of drug administration and minimizes potential side effects. Overall, ciprofloxacin-loaded gelatin microspheres offer a promising approach for enhancing drug delivery, improving patient compliance, and optimizing therapeutic outcomes in various medical applications [23-26].

Table 2. Bioactive scaffolds for wound repair and regeneration

Types of scaffold	Advantages	Disadvantages	Future prospects
Porous scaffolds	A high porosity scaffold offers a microenvironment for extracellular matrix secretion and nutrient supply to the cells. Pore sizes specific to the cell types prevent the clustering of the cells, thus avoiding necrotic center formation. Pores can be used for encapsulation of drugs and bioactive molecules	The porous nature limits the homogenous distribution of the cells. Different pore sizes are required for the specific cell types and tailoring the scaffold, which is time-consuming.	Improvement in the interconnectivity of pores and thereby the biomimetic structure of the scaffolds is required.
Fibrous scaffolds	Highly microporous structure is best suitable for cellular processes. Low inflammatory response upon implantation	Surface functionalization is required to create the nanofibers of these scaffolds.	Drugs and biological molecules such as proteins, genes, growth factors, etc., can be incorporated in fibrous scaffolds for release applications.

Table 2 (Continued)

Types of scaffold	Advantages	Disadvantages	Future prospects
Hydrogel scaffolds	Highly biocompatible and controlled biodegradation rate.	Limited mechanical strength due to soft structures.	The degradation behavior of the hydrogels and tenability should be well-defined. Hydrogels incorporate growth factors to facilitate cell differentiation.
Microsphere scaffolds	Easily fabricated with controlled physical characteristics suitable for slow or fast drug delivery. Provides enhanced cell attachment and migration properties.	Microsphere sintering methods are sometimes not compatible with the cells and reduce cell viability.	These scaffolds can be used as a target-specific delivery vehicle for drugs such as antibiotics, anti-cancer, etc.
Acellular scaffolds	The native extracellular matrix is retained and thus normal anatomical features are maintained. Less inflammatory and immune response with higher mechanical strength.	Incomplete decellularization is required to avoid immune responses.	Such scaffolds hold promise for developing artificial organs.
Composite scaffolds	Highly biodegradable and offer mechanical strength. Greater absorbability.	Acidic byproducts are generated upon degradation. Poor cell affinity. Tedious efforts in developing composite scaffolds.	Nano-bioceramic and polymer composites with faster degradation are currently being developed.

Table 3. Characteristics and applications of bioactive scaffolds

Scaffold type	Methods	Types of loaded microspheres	Characteristics of the scaffold	Applications	References
Microspheres fish-based collagen scaffold	Freeze drying	bFGF-loaded PLGA microspheres	An interconnected porous structure in the scaffold. The distribution of the microspheres in the scaffold is uniform.	Skin tissue regeneration in tissue engineering of skin	[27]
Cell culture sheet with PLGA microspheres	Culturing human fibroblasts and keratinocytes	PLGA microspheres	Cell culture on the PLGA microspheres	Skin wound healing	[28]
Porous collagen/ cellular nanocrystal scaffold		bFGF-loaded biodegradable gelatin microspheres		Skin tissue engineering	[29]
Porous chitosan−gelatin scaffold		Biodegradable chitosan−gelatin microspheres	Porous three-dimensional scaffolds	Skin regeneration and vascularization	[30]
Hybrid scaffold of PLGA and PEO	Electrospinning	rhEGF and rhbFGF-loaded PLGA microspheres	Average nanofiber diameter of 280 nm for PLGA and 760 nm for PEO	Skin regeneration and tissue engineering	[31]
Porous biodegradable microcarriers	Dermal scaffold	Cultivation of human keratinocytes on the microcarriers		Skin regeneration	[33]
Collagen/chitosan dermal scaffold	Dermal scaffold	rhVEGF and gentamicin-loaded PLGA microspheres	Distinct double-layered porous and connective structure PLGA microsphere	Skin regeneration	[34]
Biomimetic scaffold	Mesenchymal stem cells loaded on the scaffold	EGF-loaded microspheres		Skin regeneration	[35]

Therefore, this review describes the development and characterization of a reconstituted collagen scaffold impregnated with ciprofloxacin-loaded gelatin microspheres, capable of delivering ciprofloxacin in a sustained release manner for soft tissue augmentation. This approach could provide an effective solution for managing wound infections by actively involving wound dressings in the healing process and reducing the frequency of routine dressing changes. Research in this area may offer insights into alleviating the challenges of infected

wound healing. Additionally, type 1 collagen, extracted from bovine tendons—a solid waste product in slaughterhouses—has been successfully utilized for fabricating scaffolds in soft tissue repair.

5. Requirement of microsphere-incorporated collagen scaffolds for dermal wound repair and regeneration

Microspheres incorporated into collagen scaffolds show promising enhancement in dermal wound repair. The keys to its development are material requirements like biocompatible collagen matrices and biodegradable microspheres carrying bioactive agents such as growth factors. Structural requirements include porosity for cell infiltration and mechanical stability for body movement. Synchronization of collagen and microsphere degradation with tissue regeneration is crucial. Biocompatibility, controlled release of bioactive agents, and promotion of angiogenesis are essential biological requirements. Manufacturing involves techniques like electrospinning and sterilization without compromising integrity. Regulatory compliance and testing, including preclinical and clinical trials, are necessary for the approval of wound-healing products by the Food and Drug Administration or the European Medicines Agency.

Microsphere-incorporated collagen scaffolds have shown great potential for dermal wound repair and regeneration. The microspheres act as carriers for bioactive molecules, growth factors, or cells, which can enhance the healing process and promote tissue regeneration. This approach provides a controlled release of therapeutic agents, promoting cell proliferation and tissue remodeling. The collagen scaffold itself provides a supportive structure that mimics the natural extracellular matrix, promoting cell adhesion, migration, and differentiation. The incorporation of microspheres further enhances the scaffold's properties by enabling targeted delivery of therapeutic agents to the wound site. Overall, the use of microsphere-incorporated collagen scaffolds offers several advantages for dermal wound repair, including improved wound healing outcomes, reduced scarring, and enhanced tissue regeneration. This innovative approach holds great promise for advancing the field of regenerative medicine and improving patient outcomes in the treatment of skin injuries.

Ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffolds hold promise in tissue engineering and drug delivery applications. Gelatin microspheres offer a versatile platform for drug encapsulation and release, while collagen scaffolds provide a biocompatible and bioresorbable matrix for cell growth and tissue regeneration. The incorporation of ciprofloxacin, a broad-spectrum antibiotic, enhances the antimicrobial properties of the scaffold system, making it suitable for wound healing and infection control. This composite material can be tailored to release ciprofloxacin in a sustained manner, ensuring prolonged therapeutic effects while minimizing systemic toxicity. The synergistic combination of gelatin microspheres and collagen scaffold provides a dual-functionality platform for localized drug delivery and tissue regeneration. Moreover, the porous structure of the collagen scaffold promotes cell infiltration and proliferation, further enhancing tissue regeneration capabilities. Overall, the integration of ciprofloxacin-loaded gelatin microspheres into a collagen scaffold offers a promising approach for developing advanced biomaterials with dual functionalities of drug delivery and tissue engineering. This innovative system has the potential to address various clinical needs, including wound care, regenerative medicine, and infection management [35-37].

6. Biofabrication of collagen scaffold

6.1. Methods for extraction and characterization of type 1 collagen

Type 1 collagen was extracted from bovine tendons using a detailed protocol from the Central Leather Research Institute in Chennai. The process involved collecting tendons, washing them thoroughly, and treating them with

solutions like sodium peroxide and trypsin, as well as enzymes like pepsin to dissolve the collagen. After several washes and purification steps, the collagen solution was prepared and tested for purity. A collagen preparation was then homogenized with Triton X-100, dried into a scaffold, and incorporated with ciprofloxacin. The amount of drug added was based on the minimum inhibitory concentration against certain bacteria. The final prepared collagen scaffold was 2 mm thick.

6.2. Fabrication of porous collagen scaffold

The porous collagen scaffolds produced are shown in **Figure 3**.

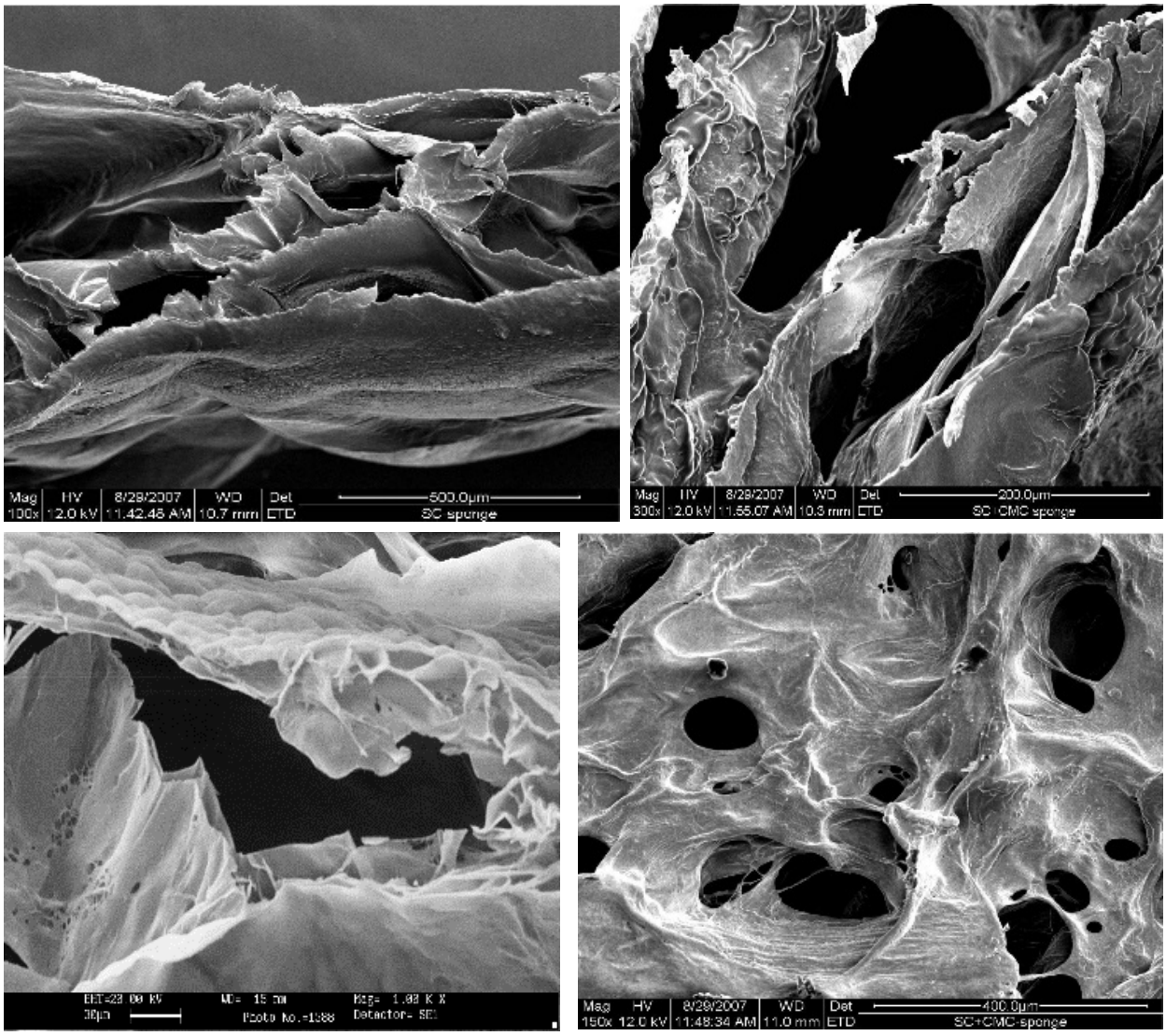


Figure 3. Porous collagen scaffold for dermal wound repair

6.3. Porous nature of collagen sponge and scaffold

The porous nature of a collagen sponge or scaffold is a critical feature that influences its performance in tissue regeneration and wound healing. Below is an overview of the key characteristics:

6.3.1. Pore structure

(1) Porosity: Collagen sponges and scaffolds are highly porous, meaning they contain numerous interconnected pores throughout their structure. The porosity typically ranges from 80% to 95%, which

is crucial for cell infiltration, nutrient transport, and waste removal.

(2) Pore size: The pore size can vary based on the fabrication technique but generally ranges between 50 μm to 500 μm. For optimal tissue ingrowth, pores need to be large enough to allow cells to migrate and proliferate while small enough to provide structural support.

(3) Surface area: The porous structure significantly increases the surface area, which enhances the scaffold's ability to support cell attachment, growth, and the delivery of bioactive agents like growth factors or antibiotics.

6.3.2. Pore interconnectivity

The interconnectivity of pores in a collagen scaffold is a crucial factor in its effectiveness for tissue regeneration and wound healing. Pore interconnectivity impacts the functionality of collagen scaffolds:

(1) Interconnected pores: In a collagen scaffold, pores are not isolated but rather form a continuous network of open spaces throughout the material. This means that each pore is connected to its neighboring pores, creating a pathway for cells, fluids, and nutrients to move through the scaffold.

(2) Fluid and nutrient exchange: Interconnected pores enable the diffusion of essential nutrients and oxygen to the cells residing within the scaffold and allow for the removal of metabolic waste products, which is critical for cell survival and proliferation.

(3) Vascularization: The porous, interconnected structure also promotes angiogenesis (the formation of new blood vessels), as endothelial cells can infiltrate the scaffold and form vascular networks.

6.3.3. Impacts on biological functions

(1) Cell infiltration: The porous structure allows cells, such as fibroblasts, keratinocytes, and endothelial cells, to penetrate the scaffold and populate its interior. This is vital for the regeneration of tissue, particularly in dermal wound repair.

(2) Degradation rate: The porous nature of the scaffold affects its degradation rate. The larger and more interconnected the pores, the faster the scaffold can be resorbed by the body as new tissue takes its place.

(3) Mechanical properties: A highly porous scaffold can sometimes sacrifice mechanical strength for higher porosity. However, the interconnectivity of pores helps distribute mechanical loads across the scaffold, providing a balance between structural integrity and flexibility, which is particularly important in areas subject to movement.

(4) Fabrication methods: (a) Freeze-drying: One of the most common techniques to create porous collagen scaffolds is freeze-drying, which results in a sponge-like structure with interconnected pores. The freezing rate and drying conditions can be manipulated to control pore size and distribution. (b) Electrospinning or three-dimensional printing: These methods can also be used to create scaffolds with precise control over pore size and distribution, ensuring uniform interconnectivity.

(5) Importance in dermal wound repair: The porous and interconnected structure of collagen scaffolds is critical for effective dermal wound healing. It allows cells to migrate, proliferate, and form new tissue while ensuring adequate vascularization and nutrient exchange. Additionally, if bioactive agents like antibiotics or growth factors are incorporated into the scaffold, the porous structure facilitates their controlled release, enhancing the healing process.

In summary, the porosity and interconnectivity of the collagen scaffold are essential features that support its role as a matrix for tissue regeneration, mimicking the natural extracellular matrix and aiding in the healing

of complex wounds.

6.4. Overview of porous collagen scaffold

A porous collagen scaffold is a promising material for soft tissue repair due to its biocompatibility, biodegradability, and similarity to the extracellular matrix. The interconnected pores within the scaffold provide a suitable environment for cell infiltration, proliferation, and tissue regeneration. Collagen, as the main protein component of the scaffold, offers structural support and promotes cell adhesion and growth. The scaffold's porous structure allows for nutrient and oxygen diffusion, waste removal, and cell-matrix interactions critical for tissue development. It also provides mechanical support during the initial stages of healing and gradually degrades as new tissue forms, avoiding the need for scaffold removal. Moreover, the scaffold can be tailored to mimic the specific properties of the soft tissue being repaired, such as skin, cartilage, or tendons, through variations in pore size, density, and composition. Overall, the porous collagen scaffold shows great potential in promoting soft tissue repair by facilitating cell migration, proliferation, and differentiation, ultimately leading to functional tissue regeneration. Its versatility and biocompatibility make it a valuable tool in tissue engineering applications.

7. Formulation of gelatin microspheres

Gelatin microspheres are prepared using the water-in-oil emulsion technique, with 7.5 wt% ciprofloxacin dissolved in 10 mL of the aqueous phase [2,5]. The incorporation of gelatin microspheres into the collagen scaffold was done by standard protocol and 0.5 g of ciprofloxacin-loaded gelatin microspheres was added to the known amount of collagen solution [4]. Drug-loaded microspheres (100 mg) were digested with 10 ml of 1N sodium hydroxide at room temperature for 12 hours. The solution was filtered and analyzed at 278 nm using high-performance liquid chromatography (HPLC), to determine the amount of ciprofloxacin present in the microspheres. The drug loading in microspheres was estimated by using the formula:

$L = Q\text{m}/W\text{m} \times 100$

Where L is the percentage loading of microspheres, Qm is the quantity of ciprofloxacin present in Wm g of microspheres. The amount of ciprofloxacin encapsulated in the microspheres was determined using the formula:

$E = Q\text{p}/Q\text{t} \times 100$

Where E is the percentage encapsulation of microspheres, Qp is the quantity of drug encapsulated in microspheres (g) and Qt is the quantity of ciprofloxacin added for encapsulation (g). The scanning electron microscope (SEM) analysis of gelatin microspheres, gelatin microsphere-incorporated collagen scaffold, and plain collagen scaffold were conducted. Differential scanning calorimetry (DSC) of ciprofloxacin and microspheres was performed using NETZSCH DSC 204. The analysis was performed with a heating range of 50–200°C and at a rate of 10°C/min.

7.1. *In vitro* release of ciprofloxacin-incorporated gelatin microspheres

In vitro release of ciprofloxacin-incorporated gelatin microspheres (5 mg) in collagen sponge was carried out at 37.1°C in phosphate-buffered saline (50 ml) at pH 7.4. The release medium was collected at predetermined time intervals and replaced with a fresh buffer of phosphate-buffered saline (1 ml) each time. The collected samples were filtered through a 0.45 mm millipore filter. The amount of ciprofloxacin released was then measured at 278 nm using a Shimadzu UV-2100 spectrophotometer and HPLC with an ultraviolet detector. The HPLC setup included a solvent delivery system (model PU-980, Jasco) and a Rheodyne injector (model 7125, Cotati). The analytical column used was a Novapak C18 cartridge with a 4-micron particle size and dimensions of 100 mm

× 8 mm internal diameter (Waters Chromatography Division), protected by a precolumn with a Novapak C18 insert. The mobile phase was composed of methanol, acetonitrile, and 0.4 M citric acid (3:1:10, v/v/v), with a flow rate of 1 mL/min at ambient temperature.

7.2. Preparation of gelatin microspheres

The preparation of the gelatin microspheres produced a good yield of 88.2%, which indicated a low loss of microspheres during preparation and recovery (**Figure 4**). The estimation of drug content and release profile is done by ultraviolet spectroscopy partially as given in **Figure 5**. The formulated microspheres were free-flowing yellow-colored powder in nature. The olive oil was found to produce a spherical microsphere without aggregation. The glutaraldehyde-saturated toluene solution was used to cross-link and stabilize the gelatin microspheres. After stabilization, the microspheres were agitated in 5 ml of 10 mM aqueous glycine solution at 37°C for 1 hour to block the residual aldehyde groups on unreacted glutaraldehyde. The stirring speed and gelatin/drug ratio were optimized by observing the particle size under a microscope.

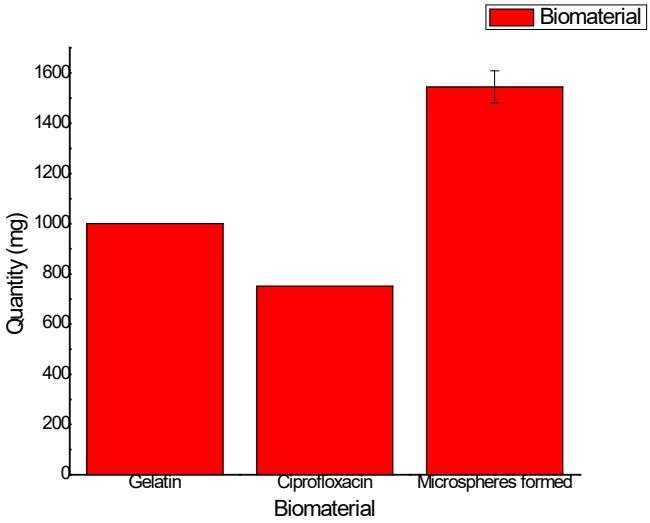


Figure 4. Quantity of microspheres formed (mg)

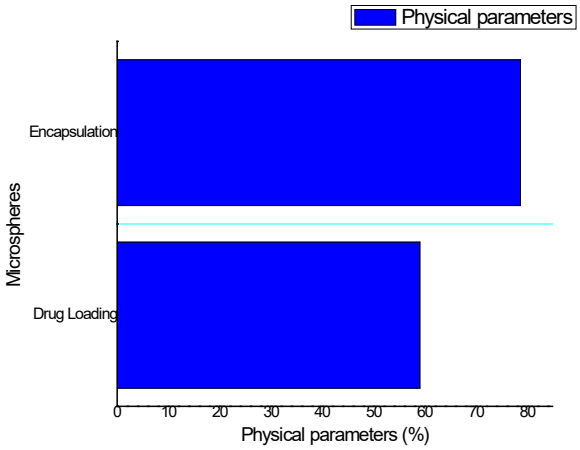


Figure 5. Physical parameters of microspheres

7.3. Scanning electron microscope study

The size and shape of ciprofloxacin-loaded gelatin microspheres were studied by SEM. As shown in **Figure 6**, the formulated microspheres were spherical and compact in nature. The particle size of the formulated gelatin microspheres was less than 50 micron as evidenced by the SEM photograph. As shown in the photograph, the particles were less aggregated and they were readily dispersed in water.

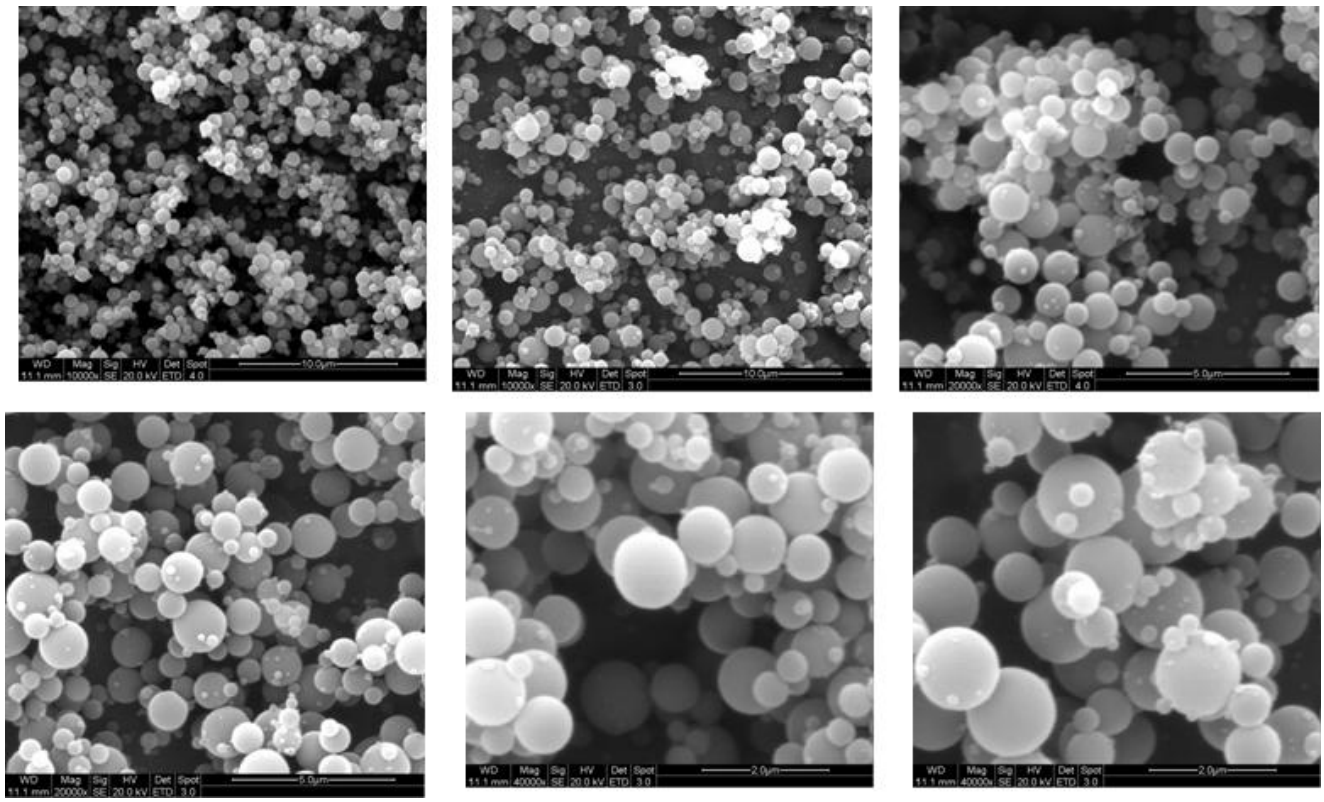


Figure 6. Ciprofloxacin-loaded gelatin microspheres

Ciprofloxacin-loaded gelatin microspheres are biodegradable drug delivery systems designed for the controlled release of antibiotics. Prepared using emulsification or solvent evaporation techniques, these microspheres encapsulate ciprofloxacin within gelatin particles. The advantages include biocompatibility, biodegradability, and drug protection. Tailoring size and composition allow for desired release rates. Studied for various applications, they show promising enhancement of therapeutic efficacy and patient outcomes.

8. Fabrication of ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffold

The fabrication of ciprofloxacin-loaded gelatin microspheres incorporated into a collagen scaffold involves a multi-step process in which drug delivery systems are designed for controlled release within a biological matrix. The general outline of the procedure is described as follows (**Figure 7**).

8.1. Preparation of ciprofloxacin-loaded gelatin microspheres

(1) Gelatin selection: An appropriate grade of gelatin is chosen depending on the desired degradation rate and biological compatibility.

(2) Drug loading: Ciprofloxacin is dissolved in a suitable solvent or buffer (e.g., water or saline). A gelatin

solution is prepared by dissolving gelatin in water, typically around 40°C. The ciprofloxacin solution is mixed with the gelatin solution thoroughly to ensure the homogenous distribution of the drug.

(3) Microsphere formation (emulsification technique): Ciprofloxacin-loaded gelatin solution is added dropwise into a non-polar solvent (such as oil) containing a surfactant (e.g., Span 80) under continuous stirring. This forms an emulsion where the aqueous gelatin phase becomes suspended in the oil. The emulsion is cooled while stirring to solidify the microspheres. The microspheres are washed several times with an organic solvent (such as isopropanol) to remove the oil phase.

(4) Crosslinking: The gelatin microspheres are cross-linked using a crosslinking agent such as glutaraldehyde to enhance stability and control degradation.

(5) Drying: The microspheres are filtered and dried, typically by freeze-drying, to maintain structure and drug stability.

8.2. Preparation of collagen scaffold

(1) Collagen extraction or purchase: Collagen can be extracted from animal tissues or purchased commercially in purified form. The collagen is dissolved in an acidic solution (such as acetic acid) to form a homogeneous gel.

(2) Scaffold formation: The collagen solution is poured into molds to form scaffolds with the desired shape and dimensions. The scaffolds are frozen to form a solid matrix, followed by lyophilization (freeze-drying) to create a porous structure, which is key for cell infiltration and tissue integration.

(3) Crosslinking: The collagen scaffold is crosslinked using a crosslinking agent like glutaraldehyde or EDC/NHS to improve mechanical properties and control the degradation rate.

8.3. Incorporation of microspheres into collagen scaffold

(1) Incorporation process: The ciprofloxacin-loaded gelatin microspheres are mixed with the collagen solution before casting, ensuring even distribution throughout the matrix. Alternatively, after the collagen scaffold is formed, the microspheres are injected or embedded into the porous structure of the scaffold.

(2) Stabilization: The scaffold-microsphere composite is frozen and lyophilized to solidify the matrix and ensure stable incorporation of the drug-loaded microspheres.

8.4. Final crosslinking and sterilization

The composite structure is further crosslinked to enhance mechanical integrity. The final product is sterilized using appropriate methods such as gamma irradiation or ethylene oxide, ensuring that the drug's potency and scaffold structure are preserved.

8.5. Characterization and evaluation

(1) Morphology: The structure is evaluated using microscopy (SEM) to confirm microsphere distribution and scaffold porosity.

(2) Drug release profile: The drug release profile of ciprofloxacin from the scaffold-microsphere composite is tested *in vitro* to ensure sustained and controlled delivery.

(3) Biocompatibility testing: The biocompatibility and cytotoxicity are assessed using relevant cell lines. This method provides a scaffold with controlled drug release properties, beneficial for tissue regeneration and infection prevention.

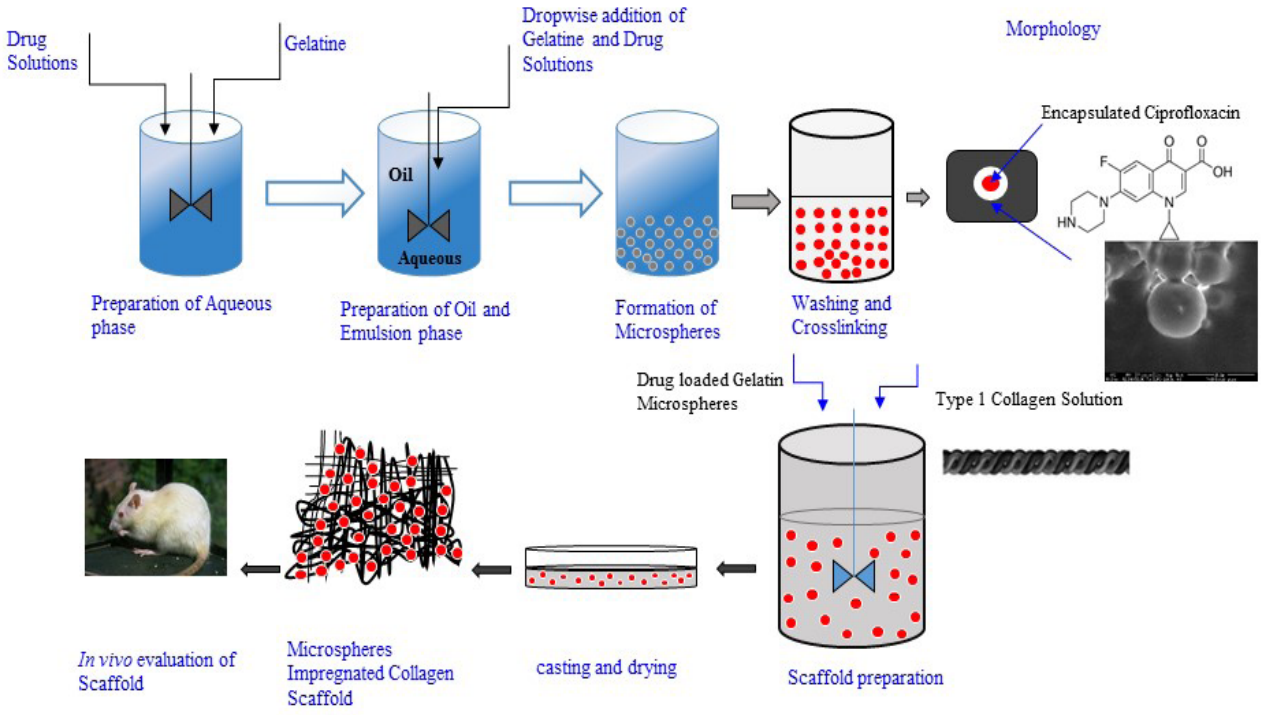
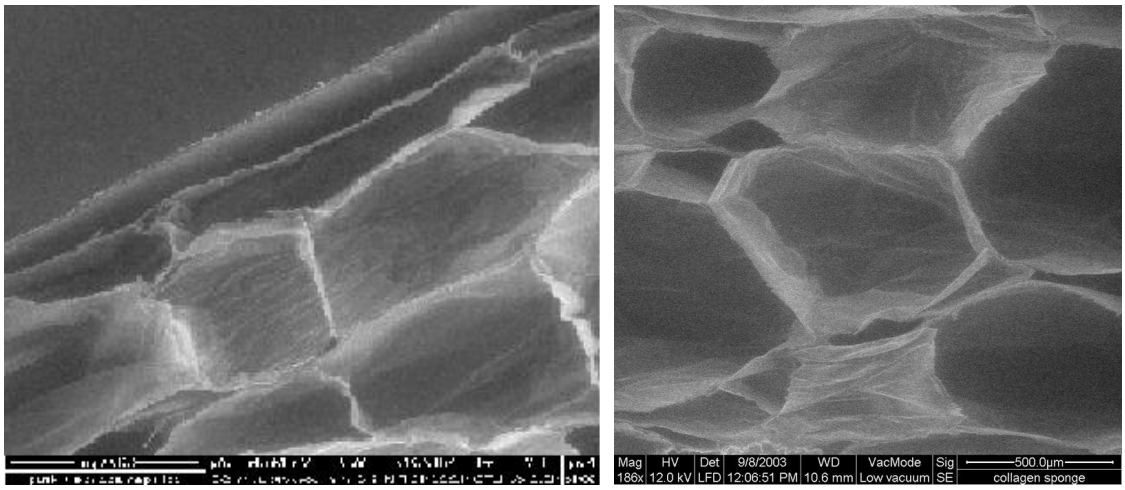


Figure 7. Fabrication of ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffold

9. Analysis of ciprofloxacin-loaded microsphere-incorporated collagen scaffolds

9.1. Characterization of collagen scaffolds

The collagen extracted from bovine tendons was used for making the scaffold. The porous scaffold has a size range of 500–700 (**Figure 8**). The pores in the scaffold help to retain the drug carrier.



Cross section of plain collagen scaffold Plain collagen scaffold

Figure 8. Plain collagen scaffold

Porous collagen scaffolds play a crucial role in tissue engineering by providing a three-dimensional environment for cell attachment and growth resembling the extracellular matrix. The scaffold's porosity influences nutrient, waste, and cell movement within it, facilitating gas exchange and transport of essential molecules for cell function. Collagen's biocompatibility and structural properties make it ideal for tissue regeneration applications. Researchers can control pore size and distribution through various fabrication techniques like freeze-

drying, electrospinning, or three-dimensional printing to tailor scaffolds for specific tissue engineering needs. The scaffold's porosity and pore size impact cell behavior and tissue formation, supporting cell migration, tissue growth, and integration with host tissues. Imaging techniques such as scanning electron microscopy or micro-computed tomography provide detailed visualization and characterization of scaffold cross-sections, aiding in understanding scaffold architecture and functionality.

Tissue engineering research and the domain of drug conveyance are a great prospering area, and the development of ciprofloxacin-loaded gelatin microspheres incorporated into a collagen scaffold is a friendly approach. The gelatin microspheres can serve as a matrix for controlled drug release to produce a sustained, localized delivery of ciprofloxacin. Adding the ciprofloxacin-loaded microspheres to a collagen scaffold provides a biocompatible and biodegradable matrix that can very well support cell growth and tissue regeneration. The combination of those materials exerts synergistic effects: for instance, the mimicry of the extracellular matrix promotes cell adhesion and proliferation via the collagen scaffold, while gelatin microspheres protect the drug payload and allow its controlled release for an extended period. Such a targeted delivery could favor improved therapeutic effects, reduced systemic side effects, and better patient compliance with ciprofloxacin treatment. This novel approach is highly promising for wound healing, tissue regeneration, and control of infection. Further research and development of these composite materials with respect to formulation and fabrication could optimize both drug delivery systems and tissue engineering strategies. The ciprofloxacin-loaded gelatin microspheres, which would be incorporated into collagen scaffolds, are highly promising in the treatment of infected dermal wounds (**Figure 9**). This is a novel strategy because two components are combined: gelatin microspheres provide successful drug delivery, while collagen scaffolds offer structural support and biocompatibility. Ciprofloxacin in gelatin microspheres could effectively target and eliminate infection in the wound site, and the collagen scaffold acts as a supportive matrix in maintaining cell adhesion, proliferation, and tissue regeneration. This combination not only helps in eradicating the infection but also encourages wound healing and tissue regeneration. The controlled release of ciprofloxacin from the gelatin microspheres within the collagen scaffold will ensure sustained therapeutic levels of the drug at the wound site for maximum efficacy with minimum systemic side effects. The fact that both gelatin and collagen are biodegradable means that, as the wound is progressively healed, the scaffold will be progressively replaced. Therefore, this hybrid approach gives promise of a new modality in the treatment of infected dermal wounds, via dual targeting of the infection and impaired process of wound healing. This will lead to better outcomes and faster recovery [35-37].

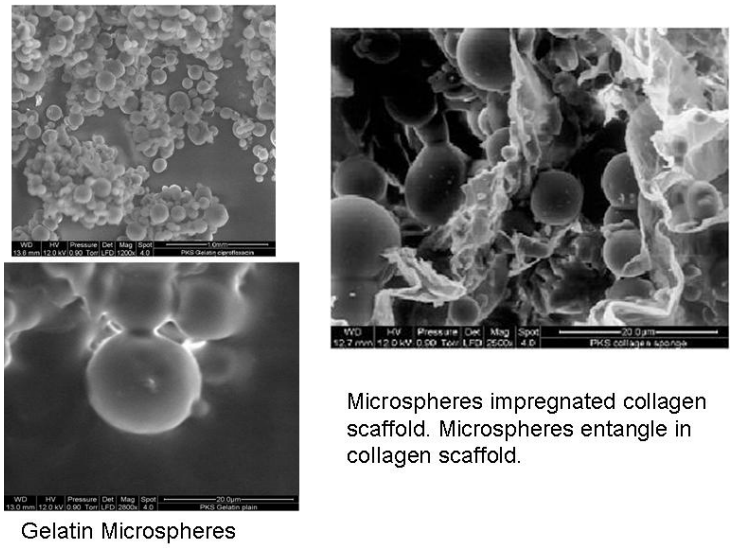
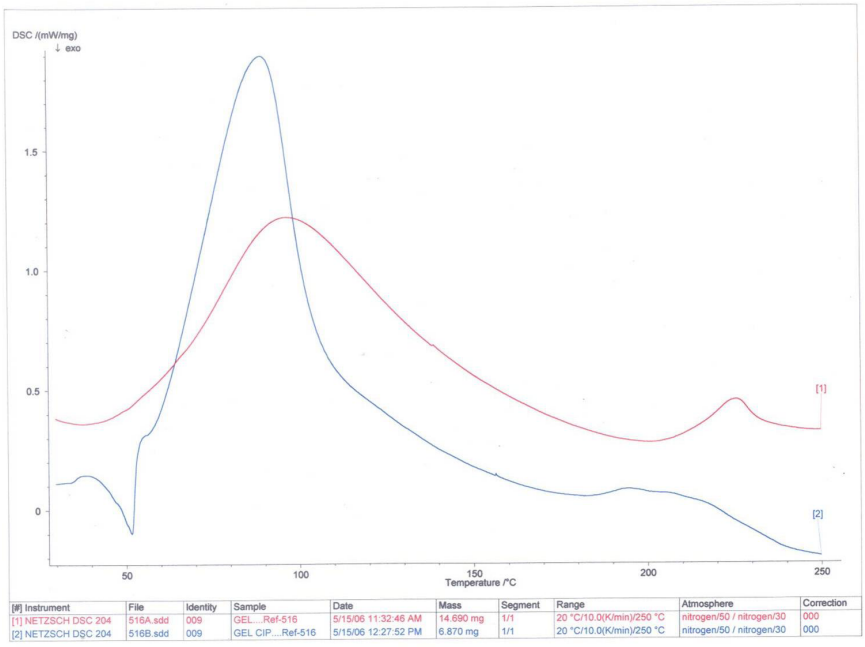


Figure 9. Gelatin microsphere-incorporated collagen scaffold

9.2. Stability of gelatin microspheres via differential scanning calorimetry analysis

Differential scanning calorimetry analysis might be a helpful tool in the evaluation of stability regarding gelatin microspheres. In DSC, the controlled heating and cooling will provide information about thermal properties comprising glass transition temperature, melting point, and crystallinity. Changes in these properties indicate changes in the physical state of gelatin microspheres, which may indicate their stability as a function of time. For instance, if there is a shift in the glass transition temperature or melting point, this could denote that the molecular arrangement of gelatin within the microspheres changes, which can influence the stability of the microspheres. Based on these thermal parameters through DSC, much essential information is deliverable regarding the structural integrity and stability of gelatin microspheres as a function of storage conditions or as different formulation changes. This information can be useful in the optimization of formulation and storage conditions to maintain stability and assure the efficacy of gelatin microspheres for different applications, including drug delivery and tissue engineering. In the present study, the DSC analysis was performed to find out the physical nature of ciprofloxacin entrapped in the gelatin microsphere and also to confirm the absence of drug–polymer interaction. The thermogram of ciprofloxacin (**Figure 10**) showed a peak at about its melting point (150.57°C). The thermogram of plain gelatin showed a peak at 96.6°C. The ciprofloxacin peak was absent in the thermogram of drug-loaded gelatin microspheres, which revealed the amorphous nature of the entrapped drug in the formulated microspheres [36,37].



[#] Instrument	File	Identity	Sample	Date	Mass	Segment	Range	Atmosphere	Correction
[1] NETZSCH DSC 204	516A.sdd	009	GEL....Ref-516	5/15/06 11:32:46 AM	14.690 mg	1/1	20 °C/10.0(K/min)/250 °C	nitrogen/50 / nitrogen/30	000
[2] NETZSCH DSC 204	516B.sdd	009	GEL CIP....Ref-516	5/15/06 12:27:52 PM	6.870 mg	1/1	20 °C/10.0(K/min)/250 °C	nitrogen/50 / nitrogen/30	000

Figure 10. DSC profile of microspheres

9.3. *In vitro* drug release profile from collagen scaffolds

The *in vitro* drug release profile of ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffolds was important for the analysis of its sustained release characteristics. The study of drug release kinetics provided an idea about the rate, duration, and mechanism through which the active agent was being released from such scaffolds. Various compositions of gelatin microspheres, collagen scaffolds, and drug-loading capacity, along with cross-linking methods, are factors that influence the profile of drug release. Such knowledge helps in understanding how those factors interact in an optimization design for targeted and controlled drug release. The various techniques of characterization that can be employed for such purposes encompass ultraviolet-

visible spectroscopy, HPLC, and SEM to observe the profile of drug release as a function of time. Besides that, they show morphology and structure in both microspheres and scaffolds. In this way, such *in vitro* drug release data can be combined with the physicochemical properties of materials to make informed decisions towards enhancing the efficiency of drug delivery and therapeutic outcomes. Gelatin microspheres are known to swell in aqueous environments due to hydration and gelatin microspheres are degraded by the presence of microbial enzymes secreted by wound pathogens and matrix metalloproteinase involved in tissue remodeling. The drug release profile shows that 27% of the drug burst released within 5 hours followed by controlled release up to 2 days (**Figure 11**).

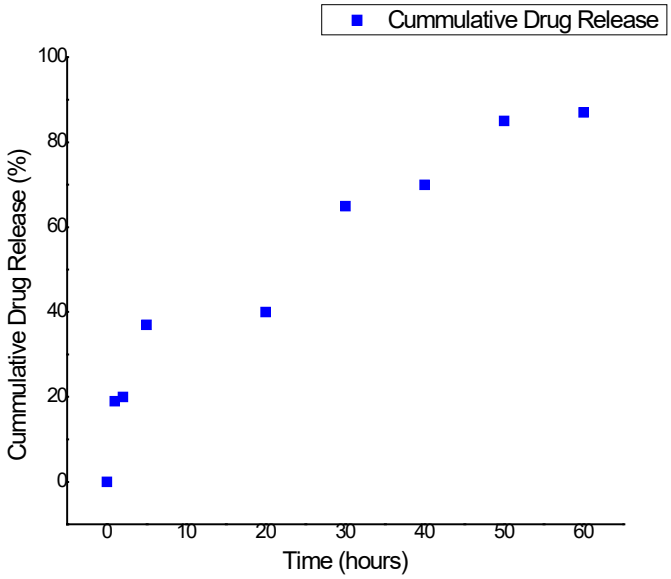


Figure 11. *In vitro* drug release of ciprofloxacin from the collagen scaffold

Ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffolds can provide both burst release and sustained release profiles of ciprofloxacin. The burst release occurs initially, where a significant amount of the drug is released quickly from the microspheres due to the drug's immediate availability on the surface or near the surface of the microspheres. This initial burst can help achieve high drug concentrations rapidly at the target site. Following the burst release, the sustained release phase begins, where the remaining drug is released slowly and continuously over an extended period. This sustained release is achieved as the drug diffuses through the gelatin matrix, which acts as a barrier to control the release rate. The collagen scaffolds further help in the controlled release of ciprofloxacin by providing a stable and supportive structure for the microspheres. By combining burst release and sustained release mechanisms, ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffolds can optimize drug delivery, providing an immediate therapeutic effect followed by a prolonged and consistent release, leading to improved efficacy and patient compliance. The activity of the released drug from the scaffold shows a clear zone of inhibition controlling the growth of bacteria inoculated in three different cultures. The drug-incorporated collagen scaffold showed a bacterial-free zone of 38 mm while the standard showed 11 mm. The initial burst release and the sustained release of ciprofloxacin are responsible for effective antimicrobial activity on the infected wounds.

9.4. *In vivo* evaluation of microsphere-incorporated collagen scaffold

9.4.1. *In vivo* evaluation of collagen scaffolds

Male Wistar albino rats weighing 150 to 200 g were used for the present study. The animals were fed with a

commercial pellet diet (Hindustan Lever, Bangalore, India) and had free access to water. The animal experiment was performed according to the Institute's ethical committee approval and guidelines (466/01/a/CPCSEA). The wound creation was done [6] and the experimental rats were dressed with formulated dressing, plain collagen scaffold, and collagen scaffold with antibiotic while the control group was dressed only with Gauss dressing (**Table 4**). All rats were given regular changes every day while the formulated dressing and the antibiotic dressing were changed once in two days.

Table 4. *In vivo* evaluation groups

Groups	Number of male Wistar albino rats (n = 96)
Open wound groups	24
Ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffold	24
Ciprofloxacin-loaded collagen scaffold	24
Plain collagen scaffold	24

9.4.2. Wound contraction

The reduction in the size of the wound was measured at every 4-day intervals and given as a percentage of wound contraction. The following formula was used to calculate the percentage of wound reduction: [(wound area day 0 – wound area day n) / wound area day 0] × 100, n = 4th, 8th, 12th, and 16th day.

The contraction of the wound is mediated by a specialized fibroblast, myofibroblast, which is found in the granulation tissue. These are known to contract collagen gel, newly synthesized in the site of healing. After an injury to the tissue, revascularization of the bed of the wound and redevelopment of the extracellular matrix is attained by cell proliferation and production of granulation tissue. Another aspect of the proliferative phase of wound healing is wound contraction, a process that takes place as a centripetal motion of tissues surrounding the wound. This increased wound contraction in the ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffold group may be associated with enhanced activities of fibroblasts. The wound contracting rate compared to the collagen scaffold and open wound groups can be compensated for the following point: subordinately, the metabolites of the microorganisms affect and suppress wound contraction and hinder the process of healing. In the ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffold group, the faster healing rate might be due to the continuous and slow release of the antibiotic. Another factor for the fast rate of healing is the presence of gelatin. The results of *in vivo* evaluation are shown in **Figure 12**.

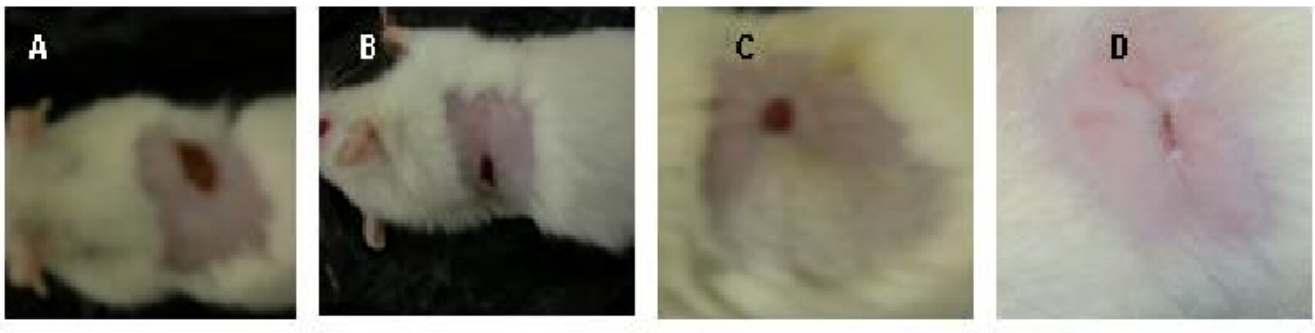


Figure 12. Wound contraction on the 12th day. (A) Open wound group. (B) Plain collagen scaffold. (C) Ciprofloxacin incorporated collagen scaffold. (D) Ciprofloxacin-loaded gelatin microsphere-incorporated collagen scaffold.

Wound contraction is a crucial phase in dermal wound healing, particularly in full-thickness wounds. It involves the movement of wound edges towards the center, reducing the wound's surface area to facilitate

closure and skin integrity restoration. This process occurs during the proliferation and remodeling phases of wound healing. Myofibroblasts are key cells involved in wound contraction, generating contractile forces through actin-myosin fibers. The extracellular matrix also plays a role by providing a structural framework that supports wound contraction. Granulation tissue formation further aids in wound contraction. While essential for healing, excessive contraction can lead to complications. Proper management and understanding of wound contraction are crucial for optimal healing outcomes.

10. Discussion

The skin, as the largest organ system in the body, serves as a protective barrier against the environment and is involved in various functions such as regulation, sensory reception, and immunological surveillance. When tissue injury occurs, the wound-healing process is automatically triggered, but infections at the wound site can impede this process. Pathogens like *Staphylococcus aureus*, *Streptococcus* species, anaerobes, and *Pseudomonas aeruginosa* are common culprits in wound infections. Biofilms formed by microbial cells can make healing more challenging by offering resistance to treatments. To address wound infections, controlled-release formulations with collagen scaffold as a carrier for drug delivery have been developed. Collagen has been found to be effective in supporting wound healing by absorbing wound fluid and providing a surface for the interaction of cells and matrices. Gelatin microspheres act as drug carriers within collagen scaffolds, helping to maintain a sustained release of antimicrobial agents at the wound site. The combination of collagen and gelatin scaffold has been shown to promote dermal regeneration and inhibit bacterial proliferation. Research has shown that collagen-based biomaterials, especially when cross-linked into a scaffold, can support cell fate processes and increase the surface area for cell-matrix interaction. By utilizing mild antigenic extracted type 1 collagen from bovine tendons, biocompatible scaffolds can be developed for tissue engineering applications. Gelatin microspheres impregnated with drugs like ciprofloxacin can be incorporated into collagen scaffolds to enhance antimicrobial activity and aid in wound healing. In wound healing, fibroblasts and myofibroblasts within granulation tissue play a crucial role in wound contraction. The presence of gelatin microspheres in collagen scaffolds has been shown to enhance fibroblast activity and promote wound contraction, leading to accelerated healing rates. The release of antibiotics from gelatin microspheres within collagen scaffolds can help prevent microbial interference, allowing for more rapid healing and dermal regeneration. Additionally, the combination of collagen scaffolds and gelatin microspheres as drug carriers shows promising wound-healing effects by providing sustained release of antimicrobial agents and enhancing fibroblast activity for wound contraction. This approach has the potential to improve the treatment of infected wounds and support dermal regeneration.

11. Conclusion

This paper discussed the development of a collagen scaffold impregnated with gelatin microspheres for controlled drug delivery on wound surfaces. The biomaterials used are natural with minimum toxicity. Collagen and gelatin have wound-healing properties. The collagen scaffold reduces dressing frequency, aids examination, and adds aesthetic value. The scaffold serves as a template for connective tissue generation in infected wounds. Collagen-based biomaterials enhance wound maturation by enabling the production of mature collagen fibers. Gelatin microspheres, impregnated in the collagen scaffold, provide controlled drug release at the wound site. Studies showed efficient healing in rats using this system, with faster healing compared to plain collagen or antibiotic-incorporated collagen scaffolds. This approach offers sustained drug release and promotes skin

regeneration. SEM was used for morphological studies, showing high encapsulation efficiency and controlled drug release over time. *In vivo* studies demonstrated accelerated wound healing with the gelatin microsphere-incorporated collagen scaffold.

Disclosure statement

The author declares no conflict of interest.

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