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Biomedical Application of Bovine Type I Collagen and Its Fabricated Scaffolds: A Review

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Abstract: The transformation of waste into wealth remains a challenging yet essential endeavor, offering opportunities for efficient solid waste management. Type I collagen, abundant in bovine tendons, serves as a valuable feedstock for the extraction of this biomaterial. Derived from slaughterhouse solid waste, bovine type I collagen acts as a foundational biomaterial for tissue engineering and regenerative medicine. This fibrous protein-based eco-material features customizable properties, including biodegradability, mechanical resilience, and surface modifiability, making it a promising alternative to synthetic and biodegradable polymers. The design and development of bioactive scaffolds remain a significant challenge in regenerative medicine, tissue engineering, and drug delivery. Collagen-based biomaterial scaffolds, which mimic the extracellular matrix, are extensively utilized as templates for tissue regeneration in biomedical applications. These scaffolds enhance wound healing and facilitate the maturation of collagen fibers, promoting the rapid formation of mature, aligned tissue at wound sites. This review provides a comprehensive analysis of the biomedical applications of collagen-based biomaterials, including their isolation and purification from bovine tendons, characterization, scaffold fabrication, ciprofloxacin/Triphala conjugation into scaffolds, biochemical and histological wound healing investigations, drug delivery, and cell culture applications. Recent advancements in chemically modified collagen and collagen-biodegradable polymer composites with controlled drug delivery for wound treatment, as well as collagen-based diffusion membranes for prolonged drug release, are also discussed.

Keywords: Bovine collagen; Ciprofloxacin/Triphala; Wound infections; Controlled release, *In vivo* studies

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1. Introduction

Biomaterials are substances used in medical devices or implants to interact with biological systems, such as tissues, organs, or bodily fluids. These materials are designed to be biocompatible and to support healing and regeneration. Examples of biomaterials include metals, ceramics, polymers, and composite materials. Their significance spans regenerative medicine, tissue engineering, and drug delivery.

Protein-based biomaterials, derived from proteins—the fundamental components of living organisms—offer versatility for applications in regenerative medicine, tissue engineering, drug delivery, and biotechnology. Examples include collagen, silk fibroin, gelatin, and elastin. These materials are biocompatible, biodegradable, and readily modifiable for specific uses. Their ability to promote cell growth and tissue regeneration underscores their value in developing advanced medical treatments and therapies.

Collagen biomaterials, derived from collagen found in animal connective tissues, are widely used due to their biocompatibility and capacity to enhance cell adhesion, proliferation, and differentiation. Their applications span wound healing, bone regeneration, cartilage repair, and drug delivery. These biomaterials can be processed into diverse forms, such as scaffolds, gels, films, and fibers, catering to various tissue engineering requirements. Advantages include natural origin, biodegradability, low immunogenicity, and ease of modification to improve mechanical properties and bioactivity. Collagen biomaterials hold significant potential for advancing medical therapies and enhancing patient outcomes [1-4].

Bovine collagen, derived from the skin, bones, and connective tissues of cows, is rich in glycine, proline, and hydroxyproline—amino acids critical for collagen production. Type I collagen from bovine tendons is notable for promoting skin elasticity, hydration, and joint health. Hydrolyzed forms of bovine collagen are commonly used in dietary supplements, cosmetics, and medical products. As the most abundant collagen type in the human body, type I collagen comprises approximately 90% of total collagen, providing structural support and flexibility to tissues like skin, bones, tendons, and ligaments. Its strength and flexibility make it integral to maintaining tissue integrity and promoting overall health [5-8].

Type I collagen biomaterials are indispensable in tissue engineering and regenerative medicine due to their biocompatibility, biodegradability, and facilitation of cell adhesion and proliferation. Applications include:

(1) Scaffolds for tissue regeneration: Supporting cell growth and differentiation in skin, bone, cartilage, and tendons.

(2) Wound healing: Creating moist environments for cell migration and reducing inflammation.

(3) Drug delivery systems: Encapsulating and releasing therapeutic agents in a controlled manner.

(4) Cosmetic applications: Improving skin elasticity and reducing wrinkles.

These attributes make type I collagen biomaterials versatile and essential for advancing biomedical innovations [9].

This paper examines the biomedical applications of type I collagen derived from bovine tendons, emphasizing its fabrication into scaffolds and films for clinical and biomedical applications.

2. Processing and characterization of type I collagen

2.1. Processing of type I collagen from bovine tendons

Bovine tendons are a widely utilized source of type I collagen for numerous medical and cosmetic applications. Collagen, the most abundant protein in the human body, is essential for maintaining the strength and structure of tissues such as skin, bones, tendons, and ligaments. Bovine tendons are rich in type I collagen, recognized for their benefits in improving skin elasticity, promoting wound healing, and supporting joint health. Collagen derived from bovine tendons can be processed into various forms, including powders, gels, and creams, for use in skin care products, wound dressings, and dietary supplements. Utilizing bovine tendons as a collagen source is both sustainable and cost-effective, as it makes use of byproducts from the meat industry. Furthermore, bovine collagen

is biocompatible and closely resembles human collagen, making it a preferred option for medical and cosmetic applications [10].

Type I collagen is primarily found in skin, bones, tendons, and other connective tissues. The abundance of this protein in bovine tendons makes them a popular choice for its extraction. The general process for extracting Type I collagen from bovine tendons includes the following steps (**Figure 1**):

(1) Tendon preparation: Bovine tendons are washed and cleaned to remove contaminants, then cut into smaller pieces to increase the surface area for extraction.

(2) Acid treatment: The tendon pieces are treated with an acid solution, such as acetic acid or hydrochloric acid, to break down collagen fibers and solubilize the protein.

(3) Extraction: Acid-treated tendon pieces are subjected to mechanical agitation or enzymatic treatment, further breaking down collagen fibers to release collagen protein into solution.

(4) Purification: The collagen solution undergoes filtration and centrifugation to remove impurities and concentrate the collagen protein.

(5) Precipitation: The purified collagen solution is precipitated using a salt solution, such as sodium chloride or ammonium sulfate, to isolate the collagen protein.

(6) Drying: The precipitated collagen is dried to remove excess moisture, yielding powdered type I collagen.

This extracted collagen finds diverse applications in the food industry as a gelling agent, in cosmetics for skincare products, and in the medical field for tissue engineering and wound healing purposes.

A specific collagen extraction protocol developed by the Bioproducts Laboratory at the Central Leather Research Institute in Chennai, India, was implemented as follows:

(1) Cleaning and swelling: Bovine tendons sourced from a local slaughterhouse in Chennai were thoroughly cleaned with distilled water to remove residual blood. The minced tissues were washed with a non-ionic surfactant and subsequently suspended in a 2% sodium peroxide solution for swelling, followed by rinsing with distilled water.

(2) Trypsin treatment: The coagulated collagen was suspended in a phosphate buffer (pH 8.5) and treated overnight with 0.5% (w/w) trypsin. Afterward, the tissue was washed to remove the enzyme and salts.

(3) Pepsin treatment: The collagen was swollen in distilled water adjusted to pH 2.5 using HCl and treated overnight with 0.3% (w/w) pepsin. Following treatment, the tissues were washed multiple times with water to eliminate residual enzymes.

(4) Purification and centrifugation: The coagulated collagen was dissolved in Millipore water acidified to pH 3.5 using HCl, creating a pure collagen solution. Undissolved proteins were separated by centrifugation at 10,000 rpm for 30 minutes.

(5) Physicochemical characterization: The entire process was conducted at 15 ± 2°C, and the collagen's purity was verified using physicochemical characterization methods [10-12].

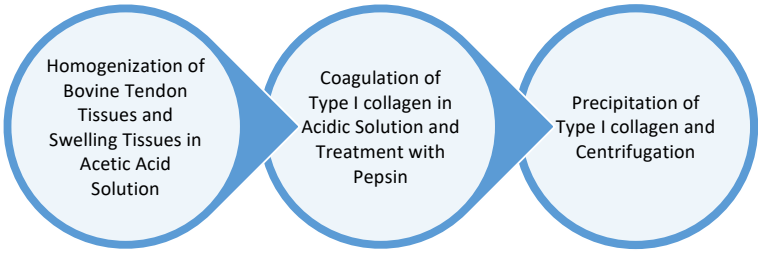

Figure 1. Processing of Type I collagen from bovine tendons

2.2. Characterization of type I collagen

The α, β, and γ chains of Type I collagen were separated based on their molecular weights using a separating gel in SDS-PAGE. The concentration of the α-1 band was observed to be twice that of the α-2 band, consistent with the known helical structure of type I collagen, which comprises three polypeptide chains: β, α1, and α2. The molecular weight of type I collagen was determined to be approximately 300 kDa. Hydroxyproline analysis revealed that about 90% of this amino acid in the extracted collagen originated from bovine tendons.

2.2.1. FTIR analysis

A prominent feature of the IR spectrum of the collagen film is the amide I band, located between 1,640–1,660 cm^{-1}, which arises from the stretching vibration of C=O (carbonyl) groups within the protein's amide groups. The strong absorption between 1,500–1,600 cm^{-1}, specifically observed at 1552.71 cm^{-1}, is attributed to the amide II mode, resulting from N-H bending coupled with the C-N stretching vibration of the amide groups. Signals within the 1,200–1,400 cm^{-1} range correspond to amide III, which arises from C-N stretching and N-H in bending within the amide linkages. The amide-A band (NH stretching), located at 3,328.26 cm^{-1}, appears almost symmetrical, indicating low water content in the sample. Additionally, the band near 1,454.83 cm^{-1} is likely associated with CH bending modes.

2.2.2. Circular Dichroism (CD) analysis

The confirmation of the triple helix structure of type I collagen was achieved through Circular Dichroism (CD) spectroscopy. The CD spectra revealed that collagen, an optically active protein, adopts a helical conformation similar to polyproline II. The spectra reveal a negative absorption band near 196 nm and a faint positive absorption band at approximately 220 nm. The collagen sample exhibited a distinct positive peak at 220 nm and a negative peak at 196 nm, confirming the presence of the characteristic triple helical structure [13-15].

3. Fabrication and functionalization of collagen scaffolds

3.1. Fabrication of porous type I collagen scaffolds for soft tissue repair

A 1% collagen solution was prepared using acetic acid in acidified water. To ensure homogeneity, 400 µL of Triton X-100, a non-ionic surfactant, was added and mixed for a few minutes. The solution was poured into a trough and allowed to dry naturally in a dust-free environment. The pore size of the collagen scaffold was measured using Scanning Electron Microscopy (SEM), as shown in **Figure 2**. The observed pores ranged in size from 500 to 600 microns [15].

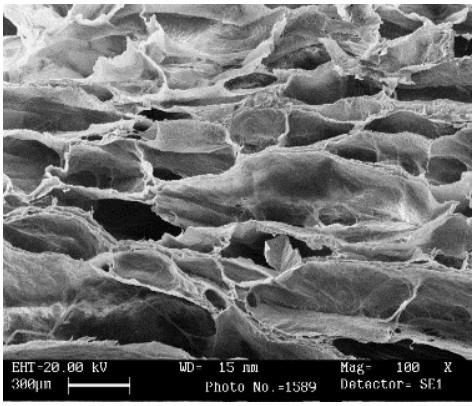

Figure 2. Pure collagen scaffold

Soft tissue repair, a common medical procedure, involves reconstructing damaged or injured tissues such as tendons, ligaments, and cartilage. Porous scaffolds fabricated from type I collagen are widely used in these applications due to their biocompatibility, biodegradability, and capacity to support tissue regeneration. A straightforward and scalable freeze-drying technique was employed to fabricate highly porous collagen scaffolds with interconnected pores. This structure closely mimics the natural extracellular matrix and provides an optimal environment for cell ingrowth and tissue regeneration.

3.1.1. Materials and equipment

(1) Type I collagen solution
(2) Distilled water
(3) Freeze-drying equipment
(4) Molds (e.g., Petri dishes or well plates)
(5) Liquid nitrogen

3.1.2. Procedure

(1) Dissolve type I collagen in distilled water as per the manufacturer's instructions, adjusting the concentration based on the desired scaffold properties.
(2) Pour the collagen solution into molds to form a thin, even layer.
(3) Place the molds in a freezer at -20°C for at least 2 hours to allow the solution to solidify.
(4) Transfer the solidified molds to freeze-drying equipment pre-cooled to -80°C.
(5) Freeze the samples at -80°C for 24 hours to ensure complete solidification.
(6) After freezing, place the samples in a vacuum chamber and apply a vacuum to remove ice crystals via sublimation, resulting in a porous collagen scaffold.
(7) Carefully remove the scaffolds from the molds after the freeze-drying process.
(8) Rinse the scaffolds with distilled water to eliminate residual chemicals or impurities.
(9) Sterilize the scaffolds using an appropriate method prior to application in soft tissue repair.

The freeze-drying method offers a simple and efficient approach for fabricating porous type I collagen scaffolds for soft tissue repair. By adjusting collagen concentration and pore size, these scaffolds can be customized to meet specific application requirements, making them a versatile solution for tissue engineering [16-18].

3.2. Conjugation of antimicrobial agents into the collagen scaffold

Conjugating antimicrobial agents into collagen scaffolds can be accomplished through various methods such as chemical modification, covalent bonding, or physical adsorption. This process involves attaching the antimicrobial agents to the collagen structure, enhancing their stability and efficacy within the scaffold. By incorporating these agents, the collagen scaffolds can exhibit antimicrobial properties, which are advantageous for applications like wound healing, tissue engineering, and drug delivery. Type I collagen is a fibrous protein that is highly susceptible to degradation by enzymes secreted by wound pathogens. Therefore, the incorporation of antimicrobial agents into collagen scaffolds was achieved to prevent microbial degradation. Additionally, this modification enables the sustained release of antimicrobial agents at the wound site to combat pathogen growth [16-18].

The urgent need for effective healing of infected skin wounds is a priority. Pathogens present in wounds release enzymes that break down surrounding tissues and form biofilms, which hinder the healing process.

Biomaterials with antimicrobial properties can aid in the regeneration of skin tissues and eliminate pathogens at the wound site. Protein-based biomaterials typically mimic the tissue at the injury site, promoting skin regeneration. Collagen, in particular, is a valuable biomaterial due to its similarity to connective tissues and its beneficial properties, such as biocompatibility, non-toxicity in most tissues, promotion of cellular growth and mobility, and a porous structure. These characteristics support the development of a well-vascularized granulation layer on the wound. Furthermore, collagen promotes the growth of keratinocytes and fibroblasts, both crucial for the healing process.

Synthetic biomaterials like polylactic glycolic acid (PLGA), polylactic acid (PLA), and polycaprolactone (PCL) are expensive and can trigger inflammatory responses in host tissues. Consequently, researchers are exploring natural biomaterials as alternatives to synthetic options.

Type I collagen is an inexpensive material derived from higher-order animals, particularly the skin, tendons, and bones, and is commonly obtained from slaughterhouses. Collagen sourced from bovine tendons contains various forms, including monomeric, dimeric, trimeric, and higher polymeric configurations (α, β, γ). Scaffolds used for wound dressings in soft tissue repair are designed to be absorbed by the body after tissue regeneration. Collagen-based scaffolds degrade naturally without causing inflammation. The porosity of these scaffolds directly affects cellular growth. The porous structure of collagen scaffolds aids in the effective encapsulation of drugs. Modern tissue engineering aims to develop three-dimensional scaffolds with biological and biomechanical properties that mimic the natural extracellular matrix, promoting tissue regeneration by allowing cell adhesion, infiltration, and proliferation for ECM synthesis. Collagen fibers are considered ideal materials for tissue engineering scaffolds due to these properties. Porous collagen scaffolds can absorb wound fluid and maintain a moist environment at the wound site. The macro porosity of collagen scaffolds efficiently encapsulates drugs and enhances cellular processes. The typical pore size of collagen scaffolds ranges from 500 to 800 microns, facilitating effective drug encapsulation (**Figure 3**).

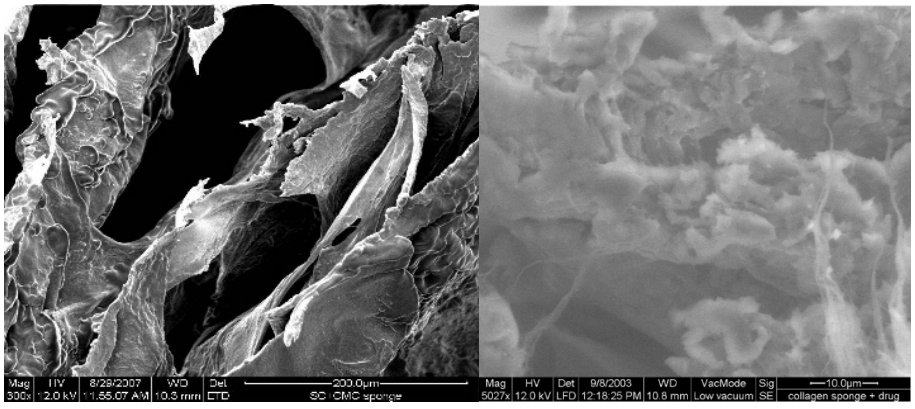

Figure 3. Ciprofloxacin incorporated collagen scaffold. **(Left)** Pure collagen scaffold with interconnectivity pores. **(Right)** Drug incorporated collagen scaffold, with the drug entrapped in the pores of the scaffold

Collagen biomaterial is generally not recommended for infected dermal wounds, as pathogens can use collagen as a growth substrate, increasing the risk of infection. Incorporating antimicrobial agents into collagen materials can mitigate this issue. An effective wound care system should deliver the appropriate amount of drug to the wound site, reduce bacterial growth, and promote dermal regeneration through sustained drug release. In collagen scaffolds incorporating ciprofloxacin, a portion of the drug is released immediately upon contact with the

wound, while the scaffold's degradation at the injury site leads to sustained drug release. The burst release of the drug demonstrates a higher minimum inhibitory concentration (MIC), inhibiting the growth of wound pathogens, as confirmed by *in vitro* antibacterial tests. *In vivo* studies indicate that the antibiotic-infused collagen scaffold group exhibits better wound closure compared to plain collagen scaffold and open wound groups.

3.3. Triphala-incorporated collagen scaffolds for soft tissue repair

The incorporation of Triphala into collagen scaffolds (**Figure 6**) has demonstrated promising results for soft tissue repair. Triphala's antioxidant properties can help reduce inflammation and promote healing. A blend of three fruits (*Emblica officinalis*, *Terminalia bellirica*, and *Terminalia chebula*), Triphala is known for its safety and lack of adverse effects, making it a favorable choice for medical applications. Adding Triphala to collagen scaffolds can enhance their biocompatibility, reducing the risk of rejection or adverse reactions when used in the body. The combination of Triphala and collagen scaffolds holds great potential for advancing soft tissue repair techniques, offering a natural and effective solution with minimal risks.

Triphala-incorporated collagen scaffolds represent a promising development in the field of soft tissue repair. These scaffolds are made of collagen, a protein that is a major component of soft tissues such as skin, muscle, and tendons. Triphala, a traditional Ayurvedic herbal formulation, has been shown to possess wound healing and anti-inflammatory properties. Collagen scaffolds provide a structure that supports cell growth and migration, both of which are essential for tissue repair. When incorporated into the collagen scaffolds, Triphala may enhance these properties by promoting collagen synthesis and reducing inflammation. Studies have shown that Triphala-incorporated collagen scaffolds can be effective in promoting the healing of dermal wounds. These scaffolds may also be useful for repairing other types of soft tissue injuries.

Some of the potential benefits of Triphala-incorporated collagen scaffolds for soft tissue repair include:

(1) Promoting collagen synthesis

(2) Reducing inflammation

(3) Supporting cell growth and migration

(4) Potential effectiveness in healing dermal wounds [17].

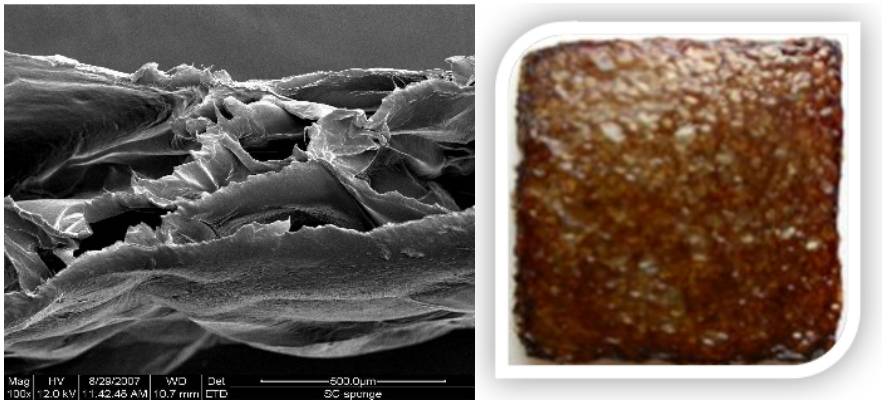

Figure 4. (Left) Pure collagen scaffold. **(Right)** Triphala incorporated collagen scaffold for soft tissue repair.

3.4. Antimicrobial agents loaded gelatin microspheres incorporated into collagen scaffolds

Recent advancements in technology have demonstrated that the use of collagen scaffolds, when coated with

antimicrobial agent-loaded gelatin microspheres, provides an effective method for combating infections in tissue engineering applications. The gelatin microspheres act as carriers, releasing the antimicrobial agents in a controlled manner. Incorporated into the collagen scaffolds, they create an environment conducive to tissue regeneration, while simultaneously being hostile to microbial growth. The antimicrobial properties help reduce the risk of infection at the implantation site and enhance integration with host tissue. The controlled release of the antimicrobial agent from the gelatin microspheres ensures prolonged protection, thereby improving the overall success of the tissue engineering process. Additionally, the excellent biocompatibility of gelatin and collagen makes this combination suitable for biomedical applications. Due to their highly porous structure, the collagen scaffolds allow for cell ingrowth and proliferation, supporting tissue regeneration. Overall, this approach represents a significant advancement in the field of tissue engineering, promoting regenerative medicine while preventing infection [16].

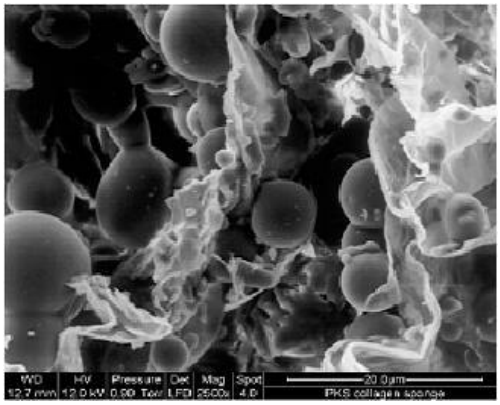

Figure 5. Ciprofloxacin-loaded gelatin microspheres impregnated collagen scaffold

Ciprofloxacin-loaded gelatin microspheres impregnated into a collagen scaffold (**Figure 5**) offer a promising method for drug delivery and tissue regeneration. These gelatin microspheres, incorporated within the collagen scaffold, enable the controlled release of ciprofloxacin, promoting localized and sustained drug delivery at the target site. The gelatin microspheres serve to protect the drug from degradation while allowing for controlled dosages. In comparison, the collagen scaffold provides a biocompatible matrix that supports cell growth and tissue regeneration. This innovative system facilitates slow ciprofloxacin release, extends treatment duration, reduces the need for high drug dosages, and enhances cell adhesion, proliferation, differentiation, and tissue healing. Consequently, ciprofloxacin-loaded gelatin microspheres impregnated into a collagen scaffold present a potential solution for localized drug delivery applications and tissue engineering, with possible applications in wound healing, tissue repair, and biomedical research [18].

4. Applications of collagen scaffolds

4.1. Tissue regeneration potential of collagen scaffolds evidenced via histology studies

Histology studies have provided strong evidence of the potential of collagen scaffolds to regenerate tissues. As the primary component of the extracellular matrix, collagen offers a biocompatible and bioresorbable matrix for tissue repair and regeneration. Histological examinations have demonstrated increased cell infiltration, proliferation, and differentiation at the site of injury or damage following treatment with collagen scaffolds. These scaffolds serve

as a structural framework that mimics the natural tissue environment, facilitating cell attachment, migration, and tissue remodeling. Additionally, collagen scaffolds have been shown to enhance angiogenesis—the formation of new blood vessels—and promote the deposition of extracellular matrix components essential for tissue healing. The collagen fibers in the scaffold not only provide mechanical support but also act as a signal to cells, promoting the regeneration of damaged tissue. In summary, histological studies suggest that collagen scaffolds induce tissue regeneration by creating a favorable microenvironment for cell activity and tissue repair processes. The evidence supports the potential of collagen-based scaffolds in tissue engineering and regenerative medicine applications.

A histological study incorporating hematoxylin and eosin (H&E) staining of granulated tissue developed in a rat model, involving treatment with collagen scaffolds for dermal wound healing, shows tissue regeneration at the cellular level. Treated Group: Histological analysis of treated tissue indicates regeneration of both the dermis and epidermis. The collagen scaffold is capable of inducing granulation tissue, characterized by the presence of fibroblasts, newly formed blood vessels (angiogenesis), and collagen deposition. The regenerated tissue shows a near-normal structure of the epidermis and dermis, with less inflammation and scar tissue formation. Untreated Group (Control): The dermis and epidermis are less organized, with less collagen deposited and incomplete tissue regeneration. Inflammation and scarring are more evident compared to the treated group. The study results show a significantly better level of wound healing with the use of collagen scaffolds in regenerating the dermis and epidermis compared to untreated wounds. Scaffolds provide a supportive environment for cell growth, enhancing tissue structure and facilitating faster healing. This study is essential for understanding how materials, such as collagen scaffolds, perform in clinical applications focused on wound repair. **Figure 6** presents H&E staining studies of granulated tissue from rats treated with collagen scaffolds for dermal wound treatment.

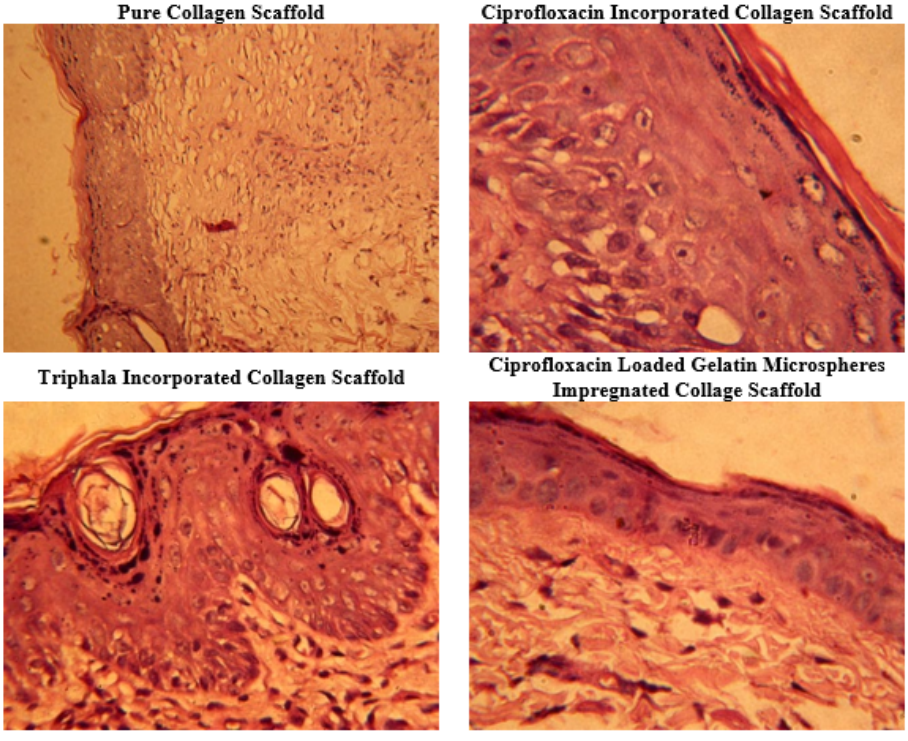

Figure 6. H&E staining studies of granulated tissue from rats treated with collagen scaffolds for dermal wound treatment

Masson's Trichrome staining is a common histological technique used to distinguish between different tissue types, particularly collagen, muscle fibers, and cell nuclei. When applied to granulated tissue from wounds in rats treated with collagen scaffolds, it provides valuable insights into collagen synthesis and tissue regeneration. **Figure 7** presents Masson's Trichrome staining studies of granulated tissue from rats treated with collagen scaffolds for dermal wound treatment. The blue or green coloration of collagen bundles confirms the presence of newly synthesized collagen fibers. Treated Group (Collagen Scaffolds): Masson's Trichrome staining highlights an abundant presence of well-organized collagen bundles in the granulated tissue of the wounds. The blue or green staining reveals extensive deposition of new collagen fibers synthesized during the healing process, indicating that the collagen scaffold promotes effective collagen production and alignment within the wound bed. The scaffold serves as a matrix that supports collagen synthesis and organization, leading to improved structural integrity and enhanced wound closure. The overall architecture of the dermal tissue appears well-formed, with distinct and dense collagen fibers, indicating a mature stage of wound healing. Untreated Group (Control): In contrast, tissue from untreated wounds shows a less organized, more sparse distribution of collagen fibers. The collagen appears disorganized, and the overall healing process is less advanced. The staining reveals less effective natural collagen synthesis without the support of the scaffold, resulting in slower or incomplete wound healing. In conclusion, Masson's Trichrome staining demonstrates that the application of collagen scaffolds in dermal wounds significantly enhances collagen synthesis. The scaffolds provide a supportive environment for the production and organization of collagen bundles, leading to more effective wound healing compared to untreated wounds. This evidence suggests that collagen scaffolds are beneficial for accelerating and improving the quality of tissue repair, particularly through their role in promoting collagen formation and alignment. This study highlights the potential of collagen scaffolds in clinical applications for dermal wound healing by improving the structural integrity and function of newly formed tissue [15-18].

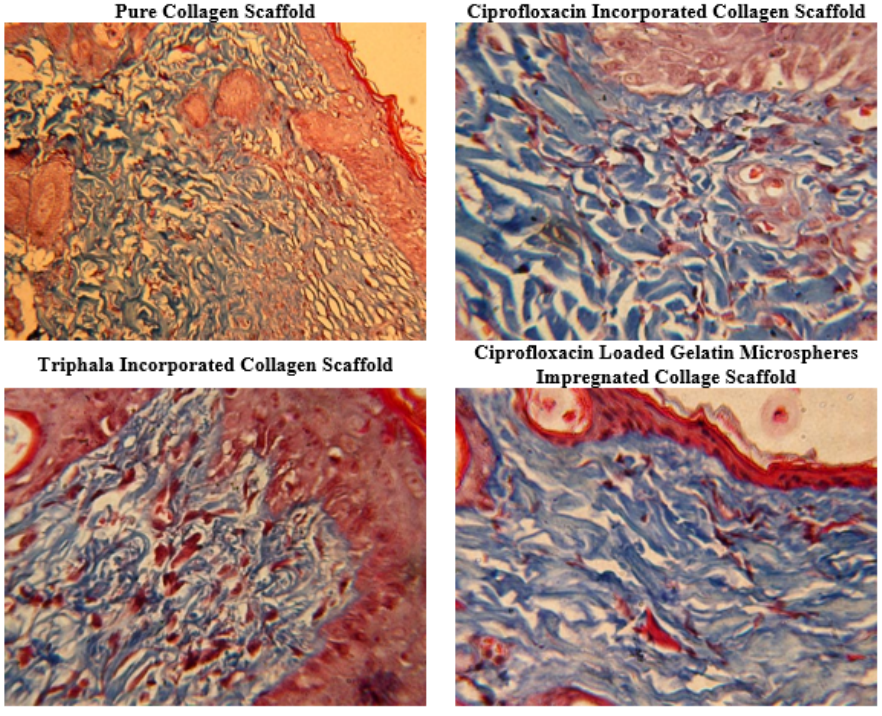

Figure 7. Masson's Trichrome staining of granulated tissue from rats treated with collagen scaffolds for dermal wound treatment

4.2. Bovine collagen for corneal application

Bovine collagen has been investigated for various biomedical applications, including corneal regeneration. It offers the advantage of biocompatibility and a composition similar to human collagen, making it an ideal component for corneal tissue engineering and wound healing. In this context, bovine collagen is utilized in corneal applications through the creation of scaffolds or implants that support the growth of corneal cells or aid in tissue repair. These scaffolds can be tailored to mimic the natural extracellular matrix of the cornea, thereby enhancing cell adhesion, proliferation, and differentiation. Bovine collagen can also serve as a carrier for bioactive molecules or drugs that promote corneal healing and regeneration. The incorporation of growth factors or antimicrobial agents into bovine collagen-based constructs allows for targeted therapeutic effects. However, concerns regarding the potential immunogenicity and disease transmission associated with bovine collagen in medical applications exist. To mitigate these risks, thorough purification and sterilization processes are essential. Further studies are required to optimize bovine collagen for corneal applications and to confirm its safety and clinical applicability. Collaboration among interdisciplinary teams of scientists, engineers, and clinicians is critical for advancing this technology and its translational potential for treating corneal diseases and injuries [19-21].

4.3. Collagen gels for dendritic cell culture

Bovine collagen gels are commonly used for culturing dendritic cells in both research and medical applications. These gels replicate the characteristics of the natural extracellular matrix, providing an environment that promotes cell adhesion, proliferation, and differentiation. Dendritic cells play a crucial regulatory role in the immune system and require an appropriate microenvironment for proper growth and function. Using bovine collagen gels for dendritic cell culture offers several advantages. The gels provide a more physiologically relevant morphology and function for dendritic cells compared to traditional culture methods. Additionally, the three-dimensional structure of the collagen gel facilitates improved cell-cell interaction and communication, which is essential for dendritic cell maturation and antigen presentation. Bovine collagen gels can also be easily modified to incorporate other components, such as cytokines, growth factors, or antigens, creating a more complex culture system for studying immune responses and therapeutic interventions. Therefore, culturing dendritic cells on bovine collagen gels offers a versatile platform for immune response studies, drug testing, and vaccine development.

4.4. Cultivation of dendritic cells in the collagen scaffold and its maturation study for the development of cell culture systems

Monocytes derived from peripheral blood were observed after 24 hours of culture in the RPMI medium. **Figure 8** illustrates the immature dendritic cells (DC).

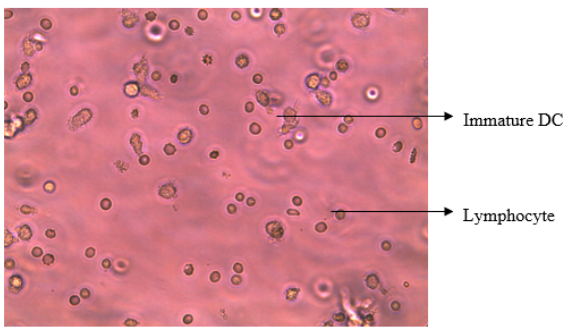

Figure 8. Dendritic cell culture observed after a 24-hour period under 40× magnification

The following images of the collagen gel (**Figures 9a–d**) were captured under 40× magnification using an inverted microscope:

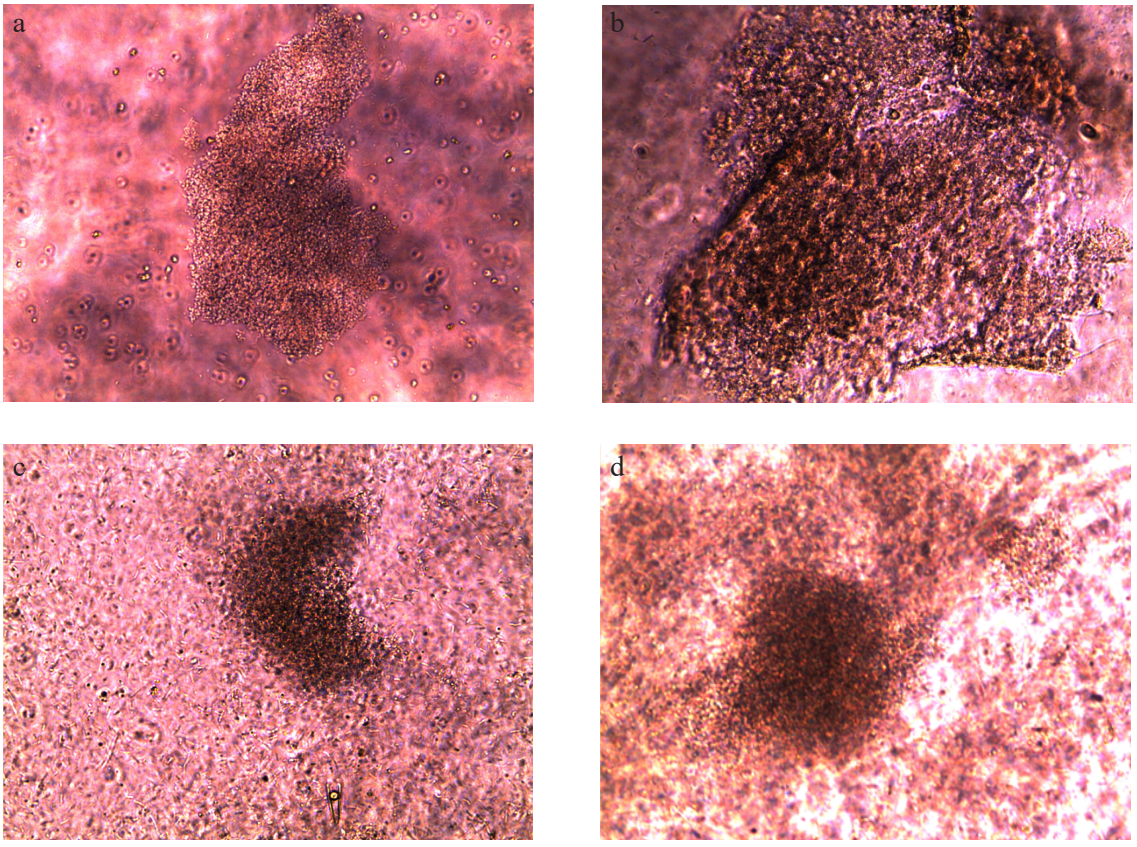

Figure 9. Growth of dendritic cells in the 3D collagen scaffold. **(a)** 10% gel (rat tail collagen); **(b)** 15% gel (rat tell collagen); **(c)** 10% gel (bovine tendon collagen); **(d)** 15% gel (bovine tendon collagen)

Type I collagen was extracted from bovine tendon, which provides an economical source for developing gels or soft scaffolds for cell culture. Collagen extracted from rat tail tendon, known for its purity, was used as an analytical-grade material for comparative studies. Recent advancements in medical technology have facilitated the development of three-dimensional DC culture systems that closely mimic *in vivo* environments. Hydrated collagen gels are widely used in 3D cell culture systems due to their structural resemblance to tissues such as the dermis and the stromal network of lymph nodes. In this study, a collagen-agarose scaffold was prepared and utilized for DC culture.

Dendritic cell maturation is a subject of significant interest due to its numerous potential applications in enhancing immune responses *in vivo*. Collagen, as an essential component of the extracellular matrix, can play a role in facilitating this maturation. In this study, DC maturation was analyzed both in the presence and absence of collagen. Without collagen, cells developed visible dendrites after 6–7 days of culture, but clusters of dead cells were observed after 10 days. In contrast, follicular dendritic cells cultured in three-dimensional collagen matrices underwent physiological maturation. Contact with type I collagen appeared to support the further maturation of immature DCs.

Culturing peripheral blood mononuclear cells (PBMCs) with granulocyte-macrophage colony-stimulating

factor (GM-CSF) in agarose or polylactic acid resulted in cell detachment from the culture plate and the formation of cell clusters, hallmarks of DC development. Notably, DCs cultured on agarose films exhibited significant detachment and visible cellular clump formation, likely due to the hydrophilic properties of the agarose. In this study, the presence of collagen facilitated both maturation and tissue aggregation in collagen-agarose gels derived from bovine and rat tail tendons. The triple-helical structure of collagen and the three-dimensional nature of the scaffold likely contributed to the integration and growth of DCs into tissue.

While it is challenging to fully exclude the influence of lipopolysaccharides (LPS) or residual collagen in the solution, the data suggest a collagen-mediated effect on DC maturation. The extraction of collagen from bovine and rat tail tendons proved to be more cost-effective than commercially available synthetic collagen and other biodegradable biomaterials. Type I collagen has demonstrated potential as a three-dimensional scaffold for cell culture systems, supporting the development of mature dendritic cell aggregates. Investigating the phenotypic changes and antigen-presenting capabilities of these mature dendritic cells on collagen scaffolds represents a compelling direction for future studies [22,23].

4.5. Collagen films for wound repair

4.5.1. Bovine type I collagen films for wound repair

Bovine type I collagen films are highly biocompatible biomaterials with properties similar to human collagen, making them effective for wound regeneration and healing. These films facilitate cell attachment, proliferation, and tissue generation, serving as scaffolds that accelerate wound closure and reduce scar formation. Additionally, they create a protective barrier against external contaminants while maintaining a moist environment conducive to the natural healing process. Collagen within these films is believed to stimulate fibroblast activity, collagen synthesis, and angiogenesis—key processes in tissue repair. As a result, the use of collagen films in wound management improves healing rates by reducing infection risk and enhancing wound closure outcomes. Their bioactive properties make them invaluable in wound care and tissue engineering applications. Ensuring the purity and quality of collagen films minimizes the risk of adverse effects or complications for patients. Proper wound care protocols should accompany the use of this biomaterial to fully realize its benefits and optimize the healing process.

Curcumin, a naturally occurring anti-inflammatory and antioxidant compound, enhances wound healing when incorporated into bovine type I collagen films. The collagen film acts as a scaffold for cellular attachment and proliferation, while curcumin promotes tissue regeneration and reduces inflammation at the wound site. Together, these elements exert a synergistic effect, accelerating the healing process. This combination can be formulated into a therapeutic film that combines the unique properties of curcumin and collagen for advanced wound care. Curcumin regulates the inflammatory response, encourages cell proliferation, and promotes angiogenesis—critical steps in effective wound healing. The collagen film provides barrier protection, prevents moisture loss, and supports tissue regeneration. Incorporating curcumin into bovine type I collagen films represents an innovative approach to wound management, with the potential to enhance healing outcomes and expand opportunities in the field of wound care.

Quercetin, a flavonoid with antioxidant and anti-inflammatory properties, also holds promise for wound healing. When incorporated into bovine type I collagen films, quercetin enhances their potential for tissue repair. The collagen film provides a scaffold for cell attachment and migration, while quercetin reduces inflammation, promotes tissue regeneration, and accelerates skin repair. This combination may improve wound closure, minimize

scarring, and facilitate rapid skin regeneration. Further studies are needed to determine the optimal dosage and formulation of quercetin for maximum therapeutic efficacy. Quercetin-loaded bovine Type I collagen films have the potential to become valuable tools in wound healing applications [24,25].

4.5.2. Phytopharmaceutical-incorporated collagen films

Soft tissue repair involves a sequence of processes, including inflammation, proliferation, and the migration of various cell types. During the acute inflammatory response, neutrophils infiltrate the wound site, producing free radicals through a "respiratory burst" activity. Non-phagocytic wound cells also generate free radicals via a NAD(P)H oxidase mechanism. Consequently, the wound environment becomes rich in reactive oxygen and nitrogen species, along with their derivatives. These radicals induce oxidative stress, leading to lipid peroxidation, DNA damage, and the inactivation of critical enzymes, including free radical scavenger enzymes.

To address oxidative stress and accelerate tissue regeneration, bioactive plant-derived compounds can be incorporated into collagen films. These films effectively scavenge free radicals produced during the wound-healing process, facilitating faster regeneration of the epidermis and dermis. Curcumin, a natural o-methoxyphenol derivative with antioxidant properties, has demonstrated potential in this context. Curcumin induces detoxification enzymes and protects against degenerative processes. When incorporated into collagen films, it provides controlled release at the wound site, supporting cellular proliferation and enhancing tissue regeneration.

Biochemical and histological analyses have confirmed that curcumin-incorporated collagen films (CICM) promote significant wound reduction by increasing cell proliferation and scavenging free radicals. These films exhibit enhanced hydrothermal stability compared to standard collagen films, maintaining their triple-helical structure, which is essential for efficacy. The application of CICM represents a promising and practical approach to dermal wound healing, combining the benefits of collagen scaffolding and curcumin's bioactivity to improve healing outcomes.

4.5.3. Quercetin-incorporated collagen matrices

Quercetin, when incorporated into collagen films, serves as a scaffold for wound repair, offering sustained release at the wound site and scavenging free radicals during the regeneration process. *In vivo* studies have demonstrated that wounds treated with quercetin-incorporated collagen showed improved healing compared to control and CS-treated wounds. These findings suggest that quercetin-incorporated collagen matrices could serve as innovative dressing materials for dermal wound healing [25].

4.6. Cardiac tissue engineering with bovine type I collagen

Collagen fibers extracted from bovine tendons can be utilized as 3D scaffolds for cardiac muscle tissue engineering. Given the limited regenerative capacity of cardiac muscle following myocardial infarction, collagen-based treatments involving cell engraftment represent a novel approach in cardiology. Collagen fleece, fabricated from type I collagen extracted from bovine tendons, possesses the mechanical strength and elasticity required for cardiomyocyte growth. This material is flexible and biocompatible, making it a promising scaffold for cultivating cardiomyocytes and developing cardiac tissue engineering constructs.

Myocardial infarction, commonly known as a heart attack, results in damage to the cardiac muscle due to decreased or interrupted blood flow. This impairment disrupts the heart's ability to pump blood effectively, as cardiac tissue lacks the intrinsic ability for self-regeneration. Studies in animal models of myocardial infarction

have demonstrated that cell engraftment improves contractile function. Recently, cardiac tissue engineering has focused on developing biomatrices or scaffolds to successfully engraft cardiac cells into the myocardium, representing a promising therapeutic strategy.

Three-dimensional scaffolds for cardiac cell culture provide an optimal microenvironment, promote cellular orientation, and facilitate cell-cell interactions. Bovine type I collagen scaffolds mimic the connective tissue structure of the human heart, incorporating a three-dimensional network of collagen fibrils. As such, this material has been employed in developing *in vitro* cardiac patches and 3D collagen fleece constructs for potential tissue engineering applications.

The structural and biochemical properties of bovine-derived type I collagen make it a leading biomaterial for cardiac tissue engineering. As a scaffold, it provides a topography that supports cell attachment, growth, and differentiation, closely mimicking the extracellular matrix environment of cardiac tissue. This facilitates the development of functional cardiac tissue constructs. Furthermore, bovine type I collagen exhibits biochemical compatibility, enabling integration between engineered tissue and host tissue upon implantation. Its angiogenic properties promote vascularization within engineered cardiac constructs, enhancing their functionality.

This versatility allows bovine type I collagen to be formed into various constructs, such as hydrogels and patches, suitable for diverse tissue engineering applications. Overall, bovine type I collagen demonstrates significant potential as a biomaterial for cardiac tissue engineering, offering a promising platform for developing cardiac patches and constructs aimed at repairing damaged heart tissue and improving cardiac function [26,27].

5. Conclusion

Type I collagen derived from bovine sources is widely utilized in various biomedical applications, including wound healing, tissue regeneration, and cardiac tissue engineering. Bioactive compounds such as Triphala, curcumin, and quercetin have demonstrated significant tissue repair and regeneration capabilities when combined with collagen-based scaffolds and films. Additionally, collagen-based scaffolds provide an optimal substrate for *in vitro* cultivation and maturation of dendritic cells. The incorporation of antimicrobial agents into collagen scaffolds has also proven effective in preventing infections and promoting healing.

Bovine type I collagen stands out as a versatile biomaterial in biomedical applications due to its biocompatibility and its capacity to mimic the natural extracellular matrix. Advancing its translational applications will require interdisciplinary collaboration among scientists, engineers, and clinicians to further enhance its potential and improve patient outcomes in clinical settings.

Disclosure statement

The author declares no conflict of interest.

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