Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 April; 6 (4s): 586 -593

# Fabrication Of Thin Tissue Grafts Using Collagen/Gelatin/Alginate Composite

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**Abstract**

**Background**: The development of biomimetic scaffolds is a primary objective in soft tissue engineering. Natural biopolymers such as collagen, gelatin, and alginate possess excellent biocompatibility but often lack the necessary mechanical stability or optimal fluid-handling capacity when utilized individually.
**Objective:** This study aims to fabricate and characterize a novel thin tissue graft utilizing a ternary collagen/gelatin/alginate composite to synergistically overcome individual material limitations for soft tissue regeneration and wound healing applications.
Methods: Composite thin films were fabricated using a solution blending and freeze-drying methodology. The resulting scaffolds were comprehensively evaluated for their microstructural architecture via scanning electron microscopy (SEM) , fluid-handling capacity via swelling ratio in phosphate-buffered saline , and chemical integration via Fourier Transform Infrared (FTIR) spectroscopy. Furthermore, antimicrobial efficacy was assessed against Escherichia coli and Staphylococcus aureus using agar well diffusion assays.
**Results:** SEM analysis confirmed a highly interconnected, porous architecture suitable for deep cellular infiltration. FTIR spectroscopy verified the successful structural integration of the constituent biopolymers, highlighting the retention of essential functional groups. The composite demonstrated a progressive swelling profile indicative of excellent wound exudate management capacity , alongside distinct, dose-dependent antimicrobial activity against both tested bacterial strains.
**Conclusion**: The blended collagen/gelatin/alginate thin films possess the requisite structural integrity, fluid-handling capacity, and bioactivity. This ternary composite demonstrates significant potential as a conformable, highly effective scaffold for soft tissue engineering and advanced wound care.

**Keywords:** Tissue engineering, Biomaterials, Collagen-gelatin-alginate, Thin tissue grafts, Wound healing, Antimicrobial scaffolds, Lyophilization.

## Introduction

Tissue engineering is a highly interdisciplinary field that aims to develop functional biological substitutes capable of restoring, maintaining, or improving damaged tissues and organs [1]. The field draws upon principles from materials science, cell biology, and biomedical engineering to construct living replacements that can integrate with host tissue and restore physiological function. A fundamental challenge within this domain is the design and fabrication of biomimetic scaffolds that accurately replicate the complex three-dimensional architecture and biochemical environment of the native extracellular matrix [2]. The extracellular matrix is not merely a static structural framework; it is a highly dynamic microenvironment that orchestrates cell adhesion, proliferation, migration, and differentiation through a complex interplay of biochemical signals, mechanical forces, and topographical cues. Consequently, an ideal tissue scaffold must fulfill multiple interrelated functions: it must provide robust mechanical support to maintain structural integrity during the early stages of healing, facilitate the mass transfer of nutrients and metabolic waste to sustain cell viability, and express the appropriate biochemical cues necessary for tissue integration and functional maturation.
In this context, the fabrication of thin tissue grafts using a ternary biopolymer composite of collagen, gelatin, and alginate has emerged as a highly promising approach for soft tissue regeneration and advanced wound healing [3]. The selection of these three biopolymers is not arbitrary; rather, it reflects a deliberate strategy to combine materials with complementary properties that together address the limitations inherent in any single component. Each biopolymer contributes distinct mechanical, biological, and processing characteristics that, when combined, yield a composite material capable of supporting cellular activity while maintaining structural integrity under physiological conditions.
Collagen, the most abundant structural protein in the mammalian extracellular matrix, is widely considered a gold standard biomaterial for tissue engineering [4]. Its native triple-helix structure provides exceptional biocompatibility, extremely low immunogenicity, and specific integrin-binding domains—such as the Arg-Gly-Asp amino acid sequence—which are critical for robust cellular attachment and subsequent intracellular signaling [5]. These integrin-mediated interactions trigger downstream signaling cascades that influence cell survival, proliferation, and differentiation, making collagen a

highly favorable substrate for supporting tissue formation. However, despite these profound biological advantages, standalone collagen scaffolds often suffer from significant limitations that restrict their clinical utility. They exhibit rapid degradation rates in vivo, often breaking down before adequate tissue regeneration can occur. Additionally, they possess inherently low mechanical strength, making them difficult to handle and prone to collapse under even modest physiological loads. Furthermore, processing pure collagen into stable, conformable structures can be challenging without the use of chemical cross-linking agents, which often introduce unwanted cytotoxicity and can alter the native biological activity of the protein [6].

To mitigate these physical processing limitations while preserving biological efficacy, gelatin is frequently incorporated into biomaterial blends. Gelatin is a natural biopolymer derived from the controlled partial hydrolysis and thermal denaturation of collagen [7]. This process breaks down the rigid triple-helix structure of native collagen, resulting in a material with markedly different physical properties. Gelatin exhibits excellent aqueous solubility, enhanced processability, and a high capacity for water absorption, making it easier to manipulate and combine with other biomaterials. Critically, gelatin retains the bioactive RGD motifs required for cell adhesion, thereby preserving the biological signaling capabilities of its parent collagen. However, gelatin possesses notoriously poor physical stability on its own; it undergoes a liquid-like phase transition at physiological temperatures around 37°C and dissolves rapidly in aqueous environments, rendering it incapable of providing long-term structural support without secondary reinforcement [8]. This temperature-sensitive behavior necessitates the incorporation of stabilizing components to maintain scaffold integrity under physiological conditions.

To confer the necessary structural integrity and fluid-handling capabilities to these proteinaceous biomaterials, structural polysaccharides are often introduced to form interpenetrating polymer networks [9]. Alginate, a naturally occurring anionic polysaccharide extracted from brown seaweed, is composed of linear copolymers containing alternating blocks of (1,4)-linked β-D-mannuronate and α-L-guluronate residues. The relative proportion and sequence of these two monomeric units influence the mechanical properties and gelation behavior of the resulting material. Alginate is extensively utilized in regenerative medicine due to its exceptional hydrophilicity and its unique ability to undergo rapid, mild gelation in the presence of divalent cations such as calcium ions. This ionic cross-linking occurs under physiological conditions without the need for organic solvents or cytotoxic reagents, creating a highly stable, water-swollen hydrogel network capable of absorbing significant amounts of fluid—a critical property for managing exudate in wound healing environments [10]. The resulting hydrogel can maintain its structural integrity for extended periods, providing a stable framework for cell infiltration and tissue formation.The fundamental rationale behind fabricating a collagen, gelatin, and alginate composite is to harness the synergistic properties of all three biopolymers while neutralizing their individual deficits. In this ternary system, alginate provides a structurally stable, moisture-retaining backbone that maintains scaffold integrity and manages fluid exudate. Native collagen provides the essential, high-fidelity biological signaling necessary for cellular infiltration, adhesion, and subsequent tissue formation. Gelatin acts as a highly processable, bioactive bridge that enhances the overall homogeneity of the blend and increases the density of cell-adhesive ligands available for interaction with infiltrating cells [11]. The combination of these three components yields a composite material whose properties exceed those achievable with any single biopolymer alone.Furthermore, fabricating this specific composite into thin tissue grafts addresses a critical bottleneck frequently encountered in soft tissue engineering: nutrient diffusion limitations. In bulky, three-dimensional scaffolds, cells located deep within the interior often suffer from hypoxia and nutrient deprivation before host vascularization can occur, leading to central necrosis and impaired tissue formation. Thin-film architectures inherently bypass this diffusion limit by reducing the distance over which oxygen and nutrients must travel to reach cells, allowing for immediate nutrient and oxygen transport throughout the scaffold. Additionally, the thin, film-like structure provides the physical flexibility, elasticity, and conformability required for applications on dynamic anatomical surfaces, such as those found in skin, oral mucosa, and other soft tissues subject to constant movement [12]. This combination of biological activity, mechanical stability, and physical adaptability makes thin-film collagen-gelatin-alginate composites highly suitable for skin tissue engineering, corneal regeneration, and the treatment of complex superficial wounds where traditional bulk scaffolds or conventional wound dressings have demonstrated limited efficacy.

**Materials and methods**

Fabrication of Composite Films

The composite films were prepared using a solution blending and freeze-drying approach. First, 0.2 g of cellulose was added to 25 mL of distilled water and stirred to initiate dispersion. Following this, 0.2 g of alginate was introduced into the cellulose suspension and mixed thoroughly to achieve a homogeneous dispersion of the polysaccharide components. In a separate preparation, 0.3 g of gelatin was dissolved in 25 mL of distilled water and heated to 50°C to facilitate complete dissolution, as gelatin exhibits improved solubility at elevated temperatures. Once fully dissolved, the gelatin solution was combined with the cellulose-alginate mixture and stirred continuously to obtain a homogeneous composite solution. The resulting mixture was then subjected to freeze-drying. The solution was first frozen and subsequently lyophilized at -80°C for 12 hours to remove water by sublimation, yielding a porous film structure.

Microstructural Characterization

Scanning electron microscopy was employed to examine the cross-sectional microstructure of the fabricated scaffolds. The instrument was operated at an accelerating voltage of 15 kV, which provided sufficient resolution to visualize the

internal pore architecture. Cross-sectional samples were prepared by carefully fracturing the films to reveal the internal structure without introducing additional artifacts.
Pore size analysis was conducted using ImageJ software. Digital images captured from scanning electron micrographs were imported into the software, and the scale was calibrated against the magnification markers embedded in the images. Pore diameters were measured across multiple representative fields of view to obtain a statistically meaningful distribution of pore dimensions within each scaffold.
Porosity Assessment
The porosity of the scaffolds was evaluated using the liquid displacement method. This technique relies on the principle that a porous material will absorb a known volume of a non-solvent liquid that does not cause swelling or dissolution of the scaffold material. Absolute ethanol was selected as the displacement liquid due to its ability to penetrate the pore network without altering the scaffold structure.
The dimensions of each scaffold were measured using a Vernier caliper to calculate the apparent volume. Prior to porosity measurement, the scaffolds were weighed to obtain their initial dry weight. Each scaffold was then submerged in absolute ethanol for 24 hours to allow complete penetration of the liquid into the pore network. After this immersion period, the scaffolds were removed, excess liquid was gently blotted from the surface, and the final weight was recorded.
All measurements were performed in triplicate for each scaffold formulation to ensure reproducibility and to account for sample-to-sample variability. Porosity was calculated using the following formula:
$$\text{Porosity} = (Wf - Wi) / (\rho \times V)$$
where Wf represents the final weight of the scaffold after ethanol immersion, Wi represents the initial dry weight of the scaffold, ρ represents the density of absolute ethanol, and V represents the apparent volume of the scaffold calculated from the Vernier caliper measurements. This method provides an indirect but reliable estimate of the void fraction within the scaffold structure.
Swelling Behavior Analysis
The swelling behavior of the fabricated scaffolds was assessed by measuring their water uptake capacity. Phosphate-buffered saline was used as the immersion medium to simulate physiological conditions. Prior to immersion, each scaffold was weighed to obtain its dry weight. The scaffolds were then submerged in phosphate-buffered saline at room temperature for a period of 2 hours. This duration was selected to allow sufficient time for the hydrophilic polymer network to reach equilibrium swelling.
After the immersion period, the scaffolds were removed from the phosphate-buffered saline, gently blotted with filter paper to remove surface-adherent liquid without compressing the scaffold structure, and weighed again to obtain the swollen weight. The degree of swelling was calculated based on the difference between the swollen weight and the dry weight, providing an indication of the scaffold's capacity to retain fluid—a property of particular relevance for wound healing applications where exudate management is critical. All swelling experiments were performed in triplicate to ensure consistency of the measurements.

**Results**

Microstructural Characterization
The surface and cross-sectional morphology of the fabricated composite scaffolds were evaluated using scanning electron microscopy. The micrographs revealed a textured surface topology characterized by an interconnected porous network extending throughout the matrix. This high degree of interconnected porosity confirms the successful sublimation of water during the freeze-drying process, which resulted in the formation of a continuous void phase without significant collapse of the polymer network. The pore architecture appeared uniformly distributed across the scaffold volume, with pore walls exhibiting smooth contours indicative of controlled ice crystal formation during the freezing stage. The observed structural architecture is highly favorable for tissue engineering applications, as it provides the necessary void fraction and continuous pathways essential for deep cellular infiltration, efficient nutrient transport, and eventual vascularization within the implanted scaffold. The interconnectivity of the pore network is particularly significant, as it ensures that cells introduced into the scaffold are not confined to the surface layers but can migrate throughout the entire structure, enabling uniform tissue formation.
Antimicrobial Activity
The composite materials demonstrated notable antimicrobial efficacy when tested against common bacterial strains using the agar diffusion method. Distinct zones of inhibition were visibly established surrounding the composite samples for both the Gram-negative Escherichia coli and Gram-positive Staphylococcus aureus cultures. The presence of these clear zones indicates that bioactive components within the scaffold matrix were released into the surrounding medium at concentrations sufficient to inhibit bacterial growth. The antimicrobial response exhibited dose-dependent characteristics, with the 100 mg composite samples producing visibly larger zones of bacterial clearance compared to the 50 mg samples across both tested strains. This dose-dependent behavior suggests a proportional release of bioactive or inhibitory components from the scaffold matrix, with higher sample masses providing a greater reservoir of antimicrobial agents available for diffusion into the surrounding environment. The observed activity against both Gram-negative and Gram-positive bacterial strains indicates that the antimicrobial mechanism is broadly effective across different bacterial cell wall architectures, a property that is particularly valuable for wound healing applications where polymicrobial colonization is

common. Swelling Behavior Analysis fluid uptake capacity, which indicates the scaffold's ability to manage wound exudate and maintain a hydrated environment conducive to tissue regeneration, was assessed by measuring water uptake capacity in phosphate-buffered saline. The swelling rate analysis demonstrated a progressive increase in fluid retention across the evaluated sample formulations designated as Samples 1 through 4. Sample 1 exhibited a baseline swelling rate of approximately 0.5, indicating a moderate capacity for fluid absorption. Sample 4 demonstrated the highest swelling capacity, exceeding a rate of 1.0, representing a substantial increase in fluid retention relative to the baseline formulation. This progressive increase in swelling capacity across the sample series suggests that modifications to the polymer composition, likely involving adjustments to the relative proportions of the hydrophilic components, enhanced the overall hydrophilicity of the scaffold matrix. This significant degree of swelling highlights the highly hydrophilic nature of the incorporated polymer network and confirms the scaffold's capacity to retain fluid, a critical property for maintaining a moist wound healing environment. Adequate fluid retention is particularly important for wound healing applications, as it prevents desiccation of the wound bed, supports cell migration, and facilitates the autolytic debridement of necrotic tissue.Chemical Characterisation Fourier Transform Infrared spectroscopy was utilized to confirm the chemical integration and structural interactions of the constituent biopolymers within the composite. This analytical technique provides information about the molecular bonding and functional groups present within the material, allowing for verification that the individual components were successfully combined without undesirable chemical degradation. The resulting spectrum displayed a broad absorption band at 3329 $cm^{-1}$, which is characteristic of the stretching vibrations of hydroxyl (-OH) and amine (-NH) groups present in the blended materials. This broad band reflects the contributions from all three biopolymers, as each contains abundant hydroxyl and amine functional groups that participate in hydrogen bonding within the composite structure.Prominent peaks observed at 1632 $cm^{-1}$ and 1554 $cm^{-1}$ correspond directly to the characteristic amide I (C=O stretching) and amide II (N-H bending) bands of the proteinaceous components. The amide I band arises primarily from carbonyl group stretching vibrations, while the amide II band results from a combination of N-H bending and C-N stretching vibrations. The presence and position of these peaks confirm that the collagen and gelatin components retained their protein secondary structure following incorporation into the composite, preserving the bioactive domains essential for cell adhesion and signaling.Additionally, the strong absorption band located at 1058 $cm^{-1}$ is indicative of the stretching vibrations of the C-O-C groups, confirming the successful integration of the polysaccharide structure. This band is characteristic of glycosidic linkages present in both alginate and cellulose, verifying that these components remained intact during the fabrication process. The combined spectral evidence confirms that the ternary biopolymer composite was successfully formed without significant chemical alteration of the individual components, preserving the functional properties of each constituent material.

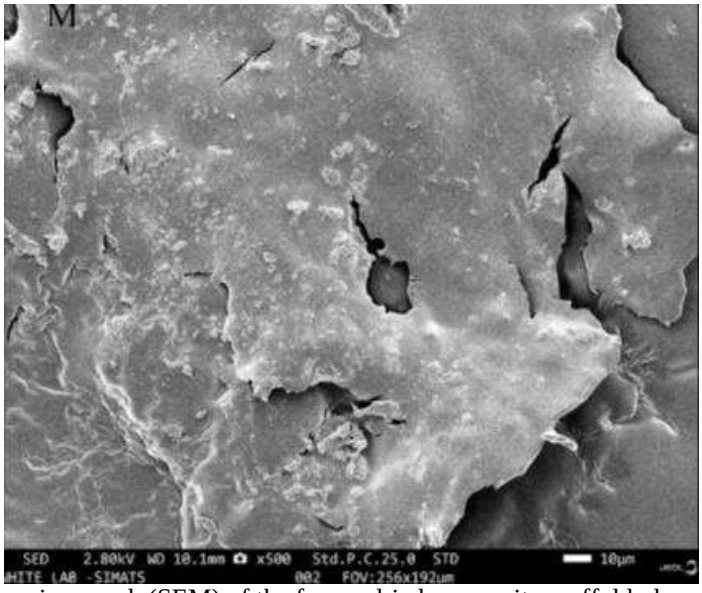


**Figure 1**. Scanning electron micrograph (SEM) of the freeze-dried composite scaffold, demonstrating a highly irregular surface morphology and interconnected porous network.

Journal for Re Attach Therapy and Developmental Diversities
eISSN: 2589-7799
2023 April; 6 (4s): 586 -593


**Figure 2.** Antimicrobial agar diffusion assay demonstrating the dose-dependent zone of inhibition against Escherichia coli (E. coli) using composite sample concentrations of 50 mg and 100 mg. 'C' denotes the control.


**Figure 3.** Antimicrobial agar diffusion assay demonstrating the dose-dependent zone of inhibition against Staphylococcus aureus (S.A) using composite sample concentrations of 50 mg and 100 mg. 'C' denotes the control.

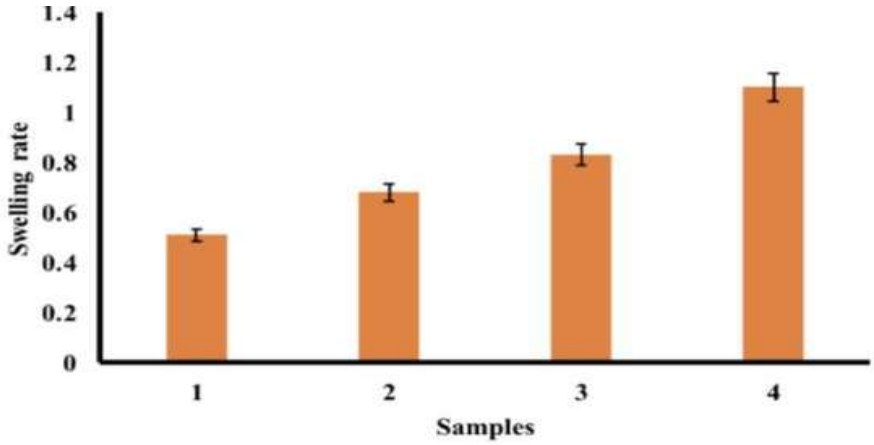


**Figure 4**. Swelling rate analysis of the composite scaffold formulations (Samples 1-4) following a 2-hour immersion in phosphate-buffered saline (PBS) at room temperature, illustrating the high fluid absorption capacity of the hydrophilic polymer network.

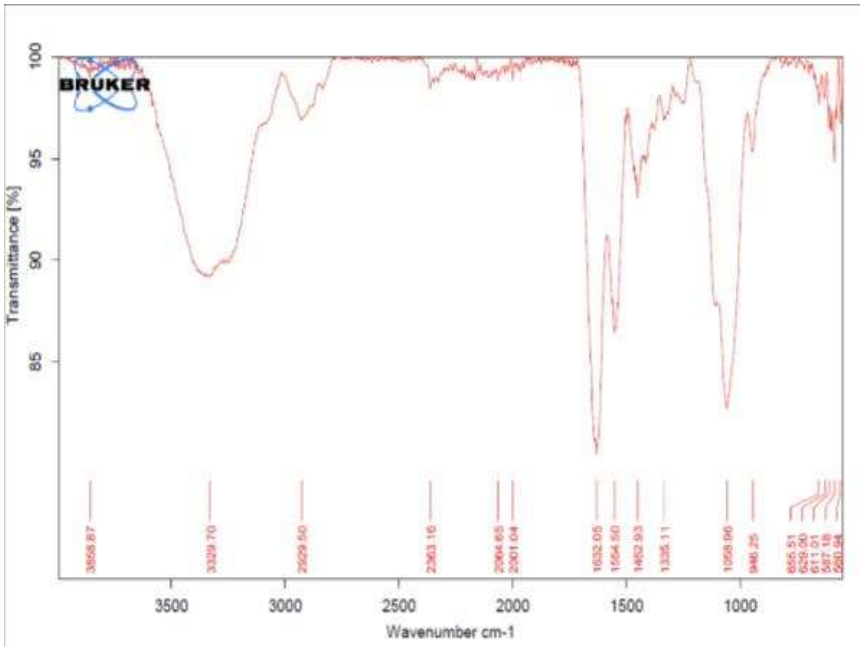


**Figure 5.** Fourier Transform Infrared (FTIR) spectrum of the composite scaffold, verifying the presence and structural integration of characteristic functional groups (amide and hydroxyl/amine bands) from the constituent biopolymers.

**Discussion**

The findings of the present study contribute to the growing body of knowledge surrounding ternary biopolymer composites for tissue engineering applications. The fabrication of thin tissue grafts using a collagen, gelatin, and alginate composite represents a promising strategy for soft tissue regeneration, as it combines the biological advantages of protein-based materials with the structural stability of polysaccharide networks. The discussion that follows interprets the observed material properties in the context of existing literature and considers the implications of these findings for the development of functional tissue replacements [13].The microstructural characteristics of the fabricated composites are consistent with observations reported in previous investigations. Gaviria and colleagues concluded that comparing the results obtained from density estimation and microstructural characterization of composites carried out by scanning electron microscopy, it could be assumed that the increment of gelatin content in the final composite led to the growth of pore isotropy that simultaneously entailed a decrease in composite density. This relationship between gelatin concentration and pore architecture is attributable to the distinct physicochemical properties of gelatin. As a denatured collagen derivative, gelatin exhibits enhanced solubility and processability compared to native collagen, which facilitates the formation of a more homogeneous polymer network during freeze-drying [14]. The increased isotropy of pore structures observed with higher gelatin content reflects the more uniform distribution of polymer material throughout the composite, resulting in a more consistent three-dimensional architecture. The corresponding decrease in density occurs because the expanded, more isotropic pore network occupies a greater proportion of the composite volume, leaving less solid material per unit volume [15].The observation that all composites exhibited very high values of total porosity, while interconnected porosity was more elevated for certain formulations, has significant implications for tissue engineering applications. High total porosity is essential for facilitating cell infiltration, nutrient transport, and waste removal—all of which are critical for supporting viable tissue formation within the scaffold. However, interconnected porosity is arguably of greater functional importance, as it ensures that the pore network is continuous throughout the scaffold, allowing cells and nutrients to penetrate into the interior rather than being confined to the surface layers [16]. The elevated interconnected porosity observed in the composite formulations suggests that the ternary blend of collagen, gelatin, and alginate creates a favorable architecture for deep cellular infiltration and vascularization, both of which are necessary for successful integration with host tissue.
The density characteristics of the polymeric blend reported in this study align with findings from previous work on similar biopolymer systems. Torres and colleagues explained that the density shown by the polymeric blend was lower than the value presented by the 100% alginate composite, probably due to the cross-linking of gelatin, which induced a less compact material [17]. This effect was expected, as the differences in the cross-linkers for both polymers—glutaraldehyde for gelatin and calcium cations for alginate—are considerable. The cross-linking mechanisms employed for each polymer influence the resulting network architecture in distinct ways. Alginate undergoes ionic cross-linking in the presence of divalent cations such as calcium, forming a hydrogel network through electrostatic interactions between the carboxylate groups of gluconate residues and the calcium ions. This cross-linking mechanism produces a relatively uniform but densely packed network. Gelatin, in contrast, is typically cross-linked through covalent bonds formed with agents such as glutaraldehyde, which creates a more loosely organized, flexible network that occupies greater volume per unit mass [18]. The combination of these two distinct cross-linking mechanisms within a single composite results in a material whose

overall density is lower than that of pure alginate, as the gelatin component introduces a less compact structural arrangement that contributes to the overall porosity of the system.The implications of these structural characteristics for tissue engineering applications are substantial. The ability to fabricate thin tissue grafts with controlled pore architecture and tunable density offers the potential to tailor scaffold properties to specific clinical applications. For wound healing applications, a highly porous, low-density scaffold can absorb significant volumes of exudate while maintaining a moist environment conducive to tissue regeneration [19]. For applications requiring greater mechanical strength, such as in load-bearing soft tissues, the scaffold architecture can be modified by adjusting the relative proportions of the constituent biopolymers to achieve the desired balance between porosity and structural integrity.The findings from this research have the potential to contribute to the development of innovative tissue engineering strategies, bringing the field closer to the goal of creating functional tissue replacements. The ability to fabricate thin tissue grafts using the collagen, gelatin, and alginate composite offers exciting prospects for regenerative medicine, where the repair and regeneration of damaged or diseased tissues could significantly impact patient outcomes and quality of life. The ternary composite approach addresses several limitations inherent in single-component scaffolds: it provides the biological signaling of native collagen, the processability and cell-adhesive properties of gelatin, and the structural stability and moisture management capabilities of alginate. By combining these properties into a single, thin-film architecture, the composite scaffold is well-suited for applications on dynamic anatomical surfaces where flexibility, conformability, and biological activity are required simultaneously [20].Future work should focus on optimizing the relative proportions of the three biopolymers to achieve the ideal balance of mechanical properties, degradation kinetics, and biological activity for specific clinical indications. Additionally, in vitro and in vivo studies are needed to evaluate the cellular response to these composite scaffolds and to assess their capacity to support tissue regeneration under physiologically relevant conditions. The incorporation of growth factors or other bioactive molecules into the composite matrix represents another promising direction for enhancing the regenerative potential of these materials. By continuing to refine the fabrication methods and material composition, it may be possible to develop clinically translatable thin tissue grafts that can address a wide range of soft tissue defects, from chronic wounds to complex surgical reconstructions.

**Conclusion**

The fabrication of thin tissue grafts using the collagen/ gelatin/alginate composite holds significant promise in various biomedical applications. The flexibility and conformability of these grafts make them ideal for situations where a tailored and adaptable scaffold is required, such as in skin tissue engineering, corneal regeneration, and cartilage repair. The ability to customise the composition and properties of the composite scaffold further enhances its potential for specific tissue regeneration applications.

The positive outcomes of this research indicate that the collagen/gelatin/alginate composite scaffold effectively supports cell attachment, proliferation, and tissue regeneration. The biomimetic properties of the scaffold contribute to the successful growth and differentiation of cells, leading to the formation of functional tissue replacements. These findings open new avenues for tissue engineering and regenerative medicine, offering potential solutions to address tissue loss or dysfunction

We concluded that the collagen/gelatin/cellulose have good biocompatible and bioactive material for wound healing applications.Continued research and refinement of the fabrication techniques, combined with thorough in vitro and in vivo evaluations, will advance the development of thin tissue grafts using the collagen/gelatin/alginate composite.

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