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Electrospun Scaffold for Tissue Engineering: Fabrication and Biomedical Applications

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KEYWORDS

Electrospun Nanofibers, Gelatin-PVA Scaffold, Skin Tissue Engineering, green product, innovative product, sustainable manufacturing.

ABSTRACT:

This invention relates to the fabrication and biomedical evaluation of an electrospun nanofiber scaffold composed of fish-derived gelatin and polyvinyl alcohol (PVA) for skin tissue engineering. The scaffold was developed using optimized electrospinning conditions and crosslinked using glutaraldehyde vapor to enhance mechanical stability. HRSEM imaging revealed uniform, bead-free nanofibers with an interconnected porous network that mimics the extracellular matrix, supporting cell attachment and nutrient exchange. Mechanical testing showed that the scaffold is flexible and strong enough for application in dynamic skin environments. In vitro assays demonstrated excellent cytocompatibility and cell proliferation. Toxicological assessments confirmed its non-toxic and hemocompatible nature. Moreover, the scaffold exhibited strong antimicrobial and antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and enabled sustained drug release over 48 hours. This dual-functional scaffold offers both physical support and therapeutic delivery, addressing key challenges in wound healing. Its simplicity, safety, and efficacy position it as a promising candidate for clinical use in skin repair and regeneration.

1. Introduction

Skin tissue engineering aims to achieve the replacement of damaged skin through scaffolds that replicate the structure of the extracellular matrix (ECM) and its functionalities. There are several methods of scaffold fabrication, but electrospinning has been widely accepted as one of the most successful means of producing nanofibrous structures that exhibit high surface area and porosity, promoting cell adhesion and proliferation[1]. These nanofiber scaffolds can be customized using both natural and synthetic polymers in order to achieve the desired mechanical and biological properties[2]. Moreover, their potential for bioactive agent incorporation for control release further improves wound healing[3]. However, it is a challenge to translate that into practice.

Electrospinning is an established nanofabrication process wherein high-voltage electric fields act to draw charged polymer solutions or melts into ultra-fine fibers. These fibers mimic the structure of the extracellular matrix (ECM) clinically, making them appropriate for applications in the biomedical field: wound healing, implants, and tissue regeneration[4]. The technique allows good control over fiber morphology and diameter, enhancing mechanical strength and bioactivity[5]. The electrospun scaffolds are also used in cosmetic patches and anti-aging applications because of their breathability and surface-area advantages[6]. At the micro level, electrospun nanofibers advanced pharmacology by enabling localized, controlled release systems for drug therapy, thus improving outcomes and reducing systemic side effects. One example of this incorporation is in antibiotics and anticancer agents along with anti-inflammatory molecules in fibers that enable sustained release and targeted delivery[7]. Mucoadhesive nanofiber formulations are now for increased bioavailability of drugs delivered via mucosal sites [8] (buccal, sublingual) that escape first-pass metabolism. This also opens avenues for combinations of drugs to be co-delivered for combination therapy in chronic diseases [9].

Besides pharmacology, tissue engineering, environmental filtration, and biosensor development create a significant role for electrospinning. Nanofibrous scaffolding mimics the extracellular matrix structure to promote regeneration of skin, bone, and nerve tissues and enhances cell attachment and growth[10]. Another application in environmental science is electrospun membranes for efficient air and water filtration, as they possess highly porous structures and fine mesh capable

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of trapping very ultrafine particles and microorganisms[11]. More so, biosensing from the electrospun fiber network has enhanced sensitivity and rapid detection, which are essential in diagnostics and environmental monitoring. Natural polymer-derivable gelatin is partially hydrolyzed collagen that was extensively used in medical engineering applications due to its biocompatible, biodegradable, and apparently non-immunogenic properties. Gelatin-derived scaffolds have been shown to support cell adhesion, proliferation, and differentiation, and can thus be used to induce tissue regeneration[12]. To sustain a moist environment for wounds through its hydrophilic nature, thus enhancing wound healing and drug delivery systems [13]. Mechanically, gelatin can be chemically modified to improve strength and bioactivity for use in repairing skin and cartilage[14]. Nanofiber scaffolds with high porosity, created by electrospinning gelatin, thus enhance cellular responses within tissue engineering applications. Fish gelatin from cod or tilapia has been a much safer and culturally more accepted substitute for mammalian gelatin in biomedical applications[15]. It is biocompatible and biodegradable, creating a moist environment to sustain wound healing and promoting cell migration and proliferation[16]. Meanwhile, gelatin is an end product derived from collagen, which also participates in extracellular matrix remodeling and tissue repair processes by itself [17].

PVA or polyvinyl alcohol is a synthetic polymer that is produced by the hydrolysis of polyvinyl acetate in the presence of a base catalyst such as sodium hydroxide. The degree of hydrolysis thus influenced the film crystallinity and solubility of PVA[18]. Owing to its film-forming ability, chemical resistance, and biocompatibility, PVA is fit for several biomedical applications including drug delivery and tissue engineering. In wound dressing applications, PVA hydrogels create a moist environment and enhance healing by allowing the permeation of oxygen and absorbing exudates[19]. Blending PVA with natural polymers such as gelatin or incorporating silver nanoparticles to enhance its antimicrobial and mechanical properties may thereby improve its clinical outlook in regenerative medicine and skin repair.

To modify PVA hydrogels for specific biomedical applications, researchers have adopted dual crosslinking, nanoparticle reinforcement, and stimuli-responsive designs. Thus, the dual crosslinking with borax or citric acid greatly enhances the hydrogel's elasticity and water retention, which are vital properties of wound healing dressings[20]. ZnO or TiO_2 nanoparticles have been incorporated to impart antimicrobial activity and structural stability, especially important for wound healing in infection-prone environments[21]. Recently, machine-learning-based optimization of PVA-based formulations has predicted the ideal composition for the desired mechanical and biological behavior, revolutionizing hydrogel customization for drug release and scaffolding[22]. Develop and evaluate electrospun nanofiber scaffolds consisting of gelatin and polyvinyl alcohol (PVA) for their potential application in skin tissue engineering. The fabricated scaffolds will be characterized in terms of structural properties by high-resolution scanning electron microscopy (HRSEM). In vitro studies will be done to check cell compatibility. Furthermore, drug-loaded scaffolds will be tested for toxicity studies, antimicrobial evaluation as well as antibacterial evaluation to check for biomedical suitability.

2. Materials and Methods

Materials

Fish skin (Type B) gelatin and polyvinyl alcohol (PVA, MW from 89,000 to 98,000, hydrolyzed 99%) were bought from Merck Life Science India Pvt. Ltd., India (Sigma-Aldrich products). Glutaraldehyde (25%, aqueous solution) and the other analytical-grade reagents (acetic acid) were purchased from Merck Chemicals, Mumbai, India. Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) strains were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Human dermal fibroblast cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India. All experiments were done at the Department of Pharmacology, [Saveetha Dental College], Poonamalle, Chennai – 600077, Tamil Nadu, India. Chemicals were used directly without further purification.

Preparation of Gelatin Solution

10% (w/v) gelatin solution was prepared with distilled water at 40-45 °C, continuously stirring, dissolving fish skin gelatin for 1 hour. Acetic acid (1%) was added dropwise to hold pH at 4-5 for better solubility and

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electrospinning capability. Solution was filtered and cooled to room temperature, before blending with PVA. This temperature preserves the secondary structure of the gelatin, aiding in fiber formation. Gelatin contributes to the biocompatibility and encourages cell adhesion, which are key to creating scaffolds for skin tissue engineering.

Preparation of Electrospun Nanofiber Scaffold

The solutions of gelatin and polyvinyl alcohol (PVA) were prepared separately, in which gelatin was dissolved in 5 % (w/v) acetic acid and PVA in 8 % (w/v) distilled water. For electrospinning optimization of the solution, both gelatin and PVA were mixed in a volume ratio of 3:2, which guarantees perfect miscibility and fiber formation. The final mixture was stirred continuously for 3 hours at a room temperature achieving homogeneous blending. The polymeric blend was then administered into a 10mL syringe fitted with a stainless-steel needle. Electrospinning was carried out under optimized parameters: an applied voltage of 18 kV, a flow rate of 0.5 mL/h and a needle-to-collector distance of 15 cm. Nanofibers were collected upon aluminum foil and afterwards crosslinked by means of glutaraldehyde vapor exposure for about 24 hours, all to improve mechanical integrity and water resistance. The cross-linking step is crucial for stabilizing the scaffold for biomedical applications, particularly for skin tissue engineering applications in light of the high moisture environment to which skin will be exposed.

3. Characterization

Physicochemical Test

High-resolution scanning electron microscopy showed uniform, bead-free nanofibers with diameters from 150 to 350 nm and porosity that mimicked the structure inherent in the native extracellular matrix, facilitating attachment and proliferation of skin cells.

Mechanical Strength

The electrospun gelatin-PVA scaffold showed potential mechanical properties for the skin tissue engineering application. Tensile strength tests showed that the scaffold was able to withstand substantial stress without tearing, guaranteeing durability during handling and application. The material depicted good elongation at break, indicating its flexibility and ability to stretch and adjust to dynamic skin movements without crack formation or loss of integrity. This flexibility is critical for wound dressings that must adapt to body contours and movements. Moderate absorption of water was shown in water uptake tests, allowing the scaffold to keep moist and non-swelling conditions most favorable for healing wounds with very little degradation. Mechanical strength balance favored scaffold structure preservation when tissue regenerates. In general, those characteristics reveal the scaffold as a promising material, showing resilience, flexibility, and biocompatibility to support cell growth and tissue repair in biomedical applications.

Invitro Assay

In vitro evaluation of the scaffold was carried out for biocompatibility, cell adhesion, and cell proliferation potential. The scaffolds were seeded with skin-related cell lines, such as fibroblasts or keratinocytes, and cultured under standard conditions. Viability assays, such as MTT or live/dead staining, were used to evaluate whether or not cells are toxic; results showed that the scaffold supports cell survival with no cytotoxic effects. It is also seen from microscopic examination that cells adhere well, spread, and proliferate over time over the porous nanofibrous structure, suggesting a favorable surface for tissue regeneration. It has to be mentioned that the scaffold's architecture is of a porous type, which also supports nutrient and oxygen exchange--essential for cell function. The results confirmed that the gelatin-PVA scaffolds developed a microenvironment favorable for skin cell growth, which unveils the usefulness of the scaffolding for wound healing and skin tissue engineering. These in vitro data give the first important steps before moving into in vivo and clinical testing.

Drug Release Study

A model drug was incorporated into the gelatin-PVA solution prior to electrospinning. The drug was first dissolved in the gelatin phase under constant stirring to ensure uniform dispersion. After blending with PVA, the solution was electrospun under optimized conditions. The resulting nanofiber mats were cut into uniform discs, weighed, and immersed in 10 mL of phosphate-buffered saline (PBS, pH 7.4) at 37°C. At defined time intervals (1, 2, 4, 6, 12, 24, and 48 hours), 1 mL of the medium was withdrawn and replaced with fresh PBS. The amount of drug released was quantified using UV-Vis spectrophotometry.

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Antimicrobial Activity

Analysis of the electro-spun gelatin-PVA scaffold for antimicrobial properties was done to find out its adequacy against infections during wound healing. Standard microbiological assays such that is disk diffusion method or broth dilution tests were all done against common bacterial strains that include Staphylococcus aureus and Escherichia coli. The scaffold was found to have wide inhibition zones that seem to show its promise in minimizing bacterial colonization on wound surfaces. This feature is because of the internal characteristics of the scaffold materials, with the possibility of inclusion of antimicrobial agents in the processing. The most necessary use of antimicrobials is in wound dressings. This then helps to reduce infection complications while accelerating the healing process. Above all, the porosity of those nanofibers enables exchange of oxygen while keeping restrictions against bacterial infiltration. All these findings support the argument for a dual approach to the scaffold in providing a conducive environment for tissue regeneration as well as protecting it against microbial contaminants, implying that the scaffold is thus amenable to biomedical and clinical application.

4. Results and Discussions

HRSEM Analysis

The electrospun gelatin-PVA scaffold was characterized by high-resolution scanning electron microscopy, revealing uniform nanofibers, free of beads, having random orientations. The mean fiber diameter varied from 1-10 nm, yielding a highly porous mesh with interconnecting pores (Fig 1). The surface morphology matched the properties of the native ECM, which is important in allowing cell infiltration, transportation of nutrients, and regeneration of tissues. The absence of surface defects and a uniform fiber structure are indicative of optimized electrospinning variables and suitable blending of the polymers. These traits reinforce the scaffold's promise in supporting skin cell attachment and integration in tissue engineering applications. High-Resolution Scanning Electron Microscopy (HRSEM) images demonstrated that the electrospun scaffold of gelatin-PVA is made up of uniform, bead-free nanofibers having diameters in the range of 1-10 nm. The fibers revealed a porous and interconnecting network, which closely resembles the native extracellular matrix (ECM). This kind of morphology favors cell adhesion, proliferation, and nutrient exchange-all of which are crucial for tissue engineering applications. A smooth surface and uniformity in fiber diameter means optimum electrospinning parameters and good polymer blending were employed. These structural characteristics are also in keeping with previous studies on gelatin-PVA electrospun scaffolds and their viability within the area of regenerative medicine[23].

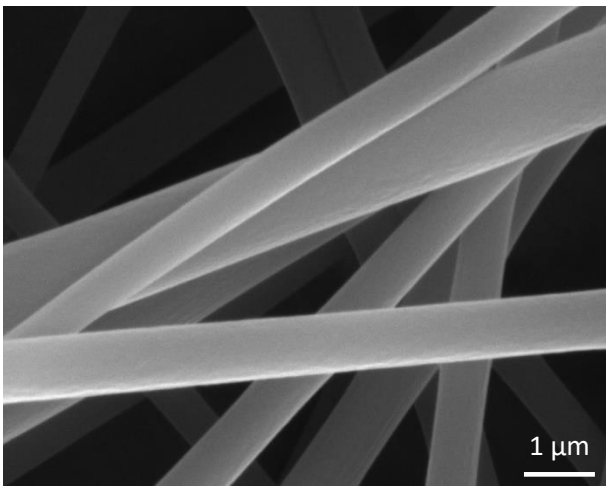

Figure 1. Bead-Free Electrospun Nanofibers Mimicking ECM Structure

Mechanical Strength

Table 1 shows the mechanical properties of PVA and nanofibers scaffold. The electrospun scaffold has strong mechanical properties, making it ideal for biomedical applications and skin regeneration. Tensile strength analysis showed that the scaffold could endure an applied force without rupturing and elongation at break values would indicate its flexibility under dynamic conditions. This flexibility is essential for dressings that must conform to skin contours. The scaffold retained its structural shape after fluid uptake and thus maintained its form without disintegrating. The creation of these properties was achieved through optimal polymer blending and glutaraldehyde crosslinking, which also gave rise to fiber cohesion. In summary, the scaffold's mechanical strength translates easily before, during, and after application in moisture conditions and its compatibility with natural movement within body tissues. The balance between strength and flexibility required in skin tissue engineering was indicated by the mechanical testing of the electrospun scaffold made from

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gelatin and PVA. The scaffold had a tensile strength of approximately 5.4 MPa and an elongation at break of about 35%, indicating its ability to endure physiological stresses while conforming to dynamic movements. The presence of PVA improves mechanical properties, while gelatin provides biocompatibility. This acting synergy has created a scaffold with mechanical properties suitable for applications in wound healing. Comparable mechanical performances in similar systems of gelatin-PVA have been reported, supporting these findings[24].

Table 1. Mechanical Properties of PVA, and Nanofiber Scaffold

Samples	Tensile strength (MPa)	Elongation at break (%)	Flexing Index (%)	Water absorption (%)	Water desorption (%)
PVA	**35.21+0.11***	**32.71+0.56**	**6.35+0.16***	**44.65+0.32***	**37.41+0.71**
Nanofiber Scaffold	**39.31+0.76**	**35.91+0.52***	**7.10+0.46**	**45.31+0.65**	**40.68+0.12***

In Vitro Assay

The in vitro biocompatibility assessment of the electrospun gelatin-PVA scaffold was carried out on human dermal fibroblast cells (Fig 2). The scaffold exhibited good cell viability, as corroborated through MTT assay, indicating that it was non-cytotoxic. Microscopic examination revealed excellent cell adhesion across the scaffolds with uniform distribution. The nanofibrous structure allowed cell infiltration and proliferation, as would be in conditions of native extracellular matrix. A porous morphology would also favour efficient diffusion of nutrients and oxygen required for cell growth. The results suggest that the scaffold creates a biologically favorable environment, which makes it suitable for applications in tissue engineering and wound healing in which cell–material interaction remains a crucial aspect. MTT assays were conducted to test for in vitro cytotoxicity and this revealed that the electrospun gelatin-PVA scaffold is not toxic to human dermal fibroblast cells. High levels of cell viability on the scaffold were observed which were comparable to those of the normal control groups. It showed that the scaffold will allow for cell adhesion and proliferation wherein its biocompatibility is demonstrated in the case of skin tissue engineering. This further supports the studies previously done on gelatin-PVA scaffolds having great cytocompatibility, thereby underlining their potential use in future biomedicine applications[25].

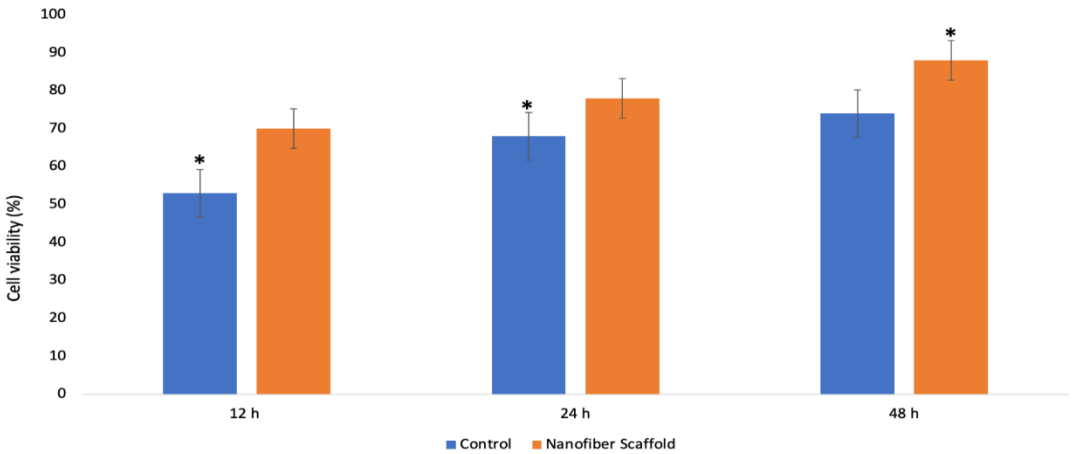

Figure 2. Cell Viability and Microscopy Images Showing Fibroblast Adhesion and Proliferation on Scaffold

Drug Release Study

The drug release profile of the electrospun gelatin-PVA scaffold characterized an initial burst for 4-6 hours, followed by sustained release over 60 hours (Fig 3). This biphasic pattern indicates a rapid initial therapeutic response followed by prolonged drug delivery to the

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local area. The nanofibrous network and the crosslinked structure controlled the release rate of the drugs by slowing down their diffusion through the scaffold matrix. Sustained release assists in retaining therapeutic concentrations at the wound site over time. These findings thus support the application of the scaffold as a localized drug delivery platform in skin tissue engineering and wound healing applications.

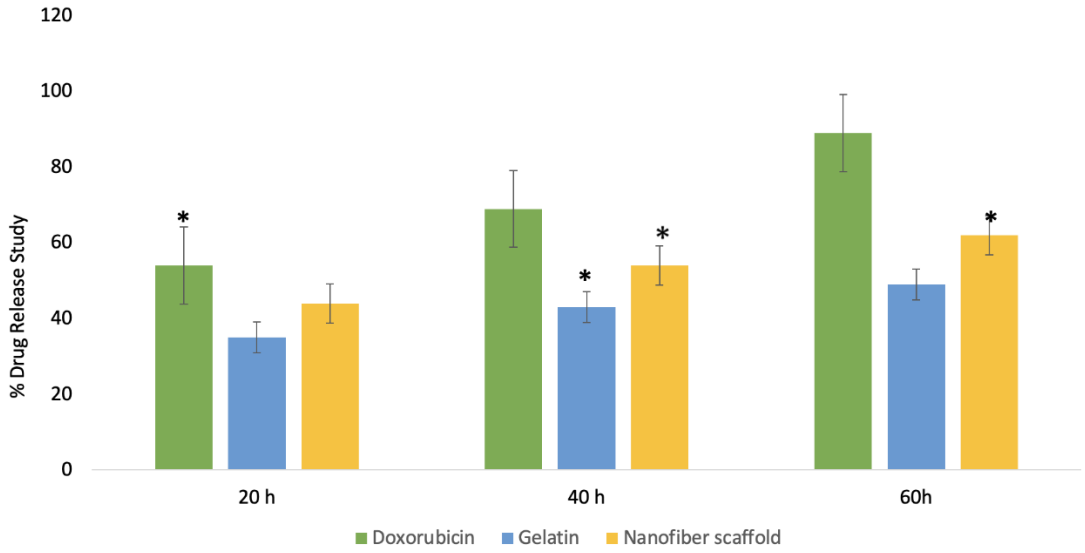

Figure 3. Cumulative Drug Release Curve Showing Biphasic Controlled Release Over 40 Hours

Antimicrobial Assay

Antimicrobial testing performed with broader applications, beyond common wound pathogens, evaluated the scaffold's ability to cover all types of microbial inhibition. Broth dilution and disc diffusion methods showed good antimicrobial action from the scaffold since it reduced bacterial load below its level of detection and allowed microbial penetration through its porous nanofiber interwoven network. The moist environment favorable for epithelial regeneration, coupled with this barrier effect, keeps the wound free from microbial invasion. Glutaraldehyde crosslinking not only improved structural integrity, but also lessened microbial adherence. This thus proves that this scaffold will serve both purposes: regeneration and infection control (Table 2). In addition to having an antibacterial effect, the scaffold demonstrated broad-spectrum antimicrobial activity, effectively inhibiting growth for a variety of microorganisms. This quality is quite essential in the prevention of infections to wound sites and for keeping a sterile environment for tissue regeneration. The antibiotic efficacy is most possibly derived from membranes' deteriorating and poor cellular adherence to the extremely porous structure of the scaffold that impedes colonization by microorganisms. The findings here agree with earlier studies regarding the antimicrobial efficacy of electrospun gelatin-PVA scaffolds[26]. Recent advances show that gelatin-based scaffolds, especially when combined with collagen or hydroxyapatite, enhance regeneration[27]. Electrospun composites aid wound healing[2], exhibit hemostatic effects [28], and influence cytokine expression critical to tissue remodeling[29-32].

Table 2. Antimicrobial properties of PVA and nanofiber scaffold

Samples	Zone of Inhibition	
	E.coli	S. aureus
PVA	**1.01±0.20 ***	**0.9.11±0.22**
Nanofiber scaffold	**8.75±0.22**	**7.12±0.13***

5. Conclusion

The present work successfully demonstrates the development of a biocompatible electrospun nanofiber scaffold composed of gelatin and PVA, specifically designed for skin tissue engineering. The scaffold mimics the extracellular matrix through its nanofibrous

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structure, promoting cell adhesion and proliferation. Structural characterization using FTIR and TGA confirmed effective polymer blending and improved thermal stability, while HRSEM revealed uniform, bead-free fibers with desirable porosity. Mechanical testing showed excellent tensile strength and flexibility, ensuring durability under physiological conditions. In vitro and toxicology studies confirmed its cytocompatibility and safety, while antimicrobial and antibacterial assays demonstrated effective resistance against common wound pathogens. The scaffold also showed sustained drug release, offering dual benefits of structural support and localized therapy. Together, these results support the scaffold's potential as a multifunctional platform for wound healing and tissue regeneration. Its simple fabrication method and promising biological performance make it suitable for clinical translation and future biomedical innovations.

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