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Fabrication and Mechanical Characterization of Electrospun Nanofiber Scaffolds for Guided Tissue Engineering in Cartilage Repair and Regeneration

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Abstract

Cartilage tissue engineering has emerged as a promising solution for repairing and regenerating damaged cartilage, overcoming the limitations of conventional treatments such as autografts and allografts. Electrospun nanofiber scaffolds have garnered significant attention due to their biomimetic structure, mechanical properties, and biocompatibility, which provide a conducive environment for chondrocyte growth and extracellular matrix (ECM) deposition. This study explores the fabrication and mechanical characterization of electrospun nanofiber scaffolds specifically designed for guided tissue engineering in cartilage repair. The electrospinning process, fiber morphology, mechanical strength, and biological performance of these scaffolds are analyzed. The study also evaluates recent advancements in scaffold modifications, including composite materials and biofunctionalization strategies. The findings contribute to the development of optimized scaffolds for enhanced cartilage regeneration.

Keywords: Electrospinning, nanofiber scaffold, cartilage tissue engineering, mechanical characterization, guided tissue engineering, biomaterials.

1. INTRODUCTION

Cartilage injuries are a significant clinical challenge due to the limited self-healing capacity of articular cartilage. The avascular nature of cartilage hinders cell migration and nutrient supply, leading to ineffective regeneration following injury. Traditional treatments such as microfracture, autologous chondrocyte implantation (ACI), and osteochondral transplantation often present drawbacks, including donor site morbidity and limited long-term efficacy.

Tissue engineering offers a promising alternative for cartilage repair by combining biomaterials, cells, and bioactive molecules to restore function and integrity. Among biomaterials, electrospun nanofiber scaffolds have gained widespread interest due to their ability to mimic the native extracellular matrix (ECM) of cartilage. Electrospinning enables the fabrication of nanofibers with high surface-area-to-volume ratios, tunable porosity, and controlled fiber alignment, which can influence cell adhesion, proliferation, and differentiation.

This study focuses on the fabrication and mechanical characterization of electrospun nanofiber scaffolds intended for cartilage tissue engineering applications. The mechanical properties of these scaffolds are crucial for maintaining structural integrity and supporting chondrocyte function. By optimizing scaffold composition and fabrication parameters, electrospun scaffolds can enhance cell-scaffold interactions and improve cartilage regeneration outcomes.

2. Literature Review

The field of cartilage tissue engineering has seen remarkable advancements in the fabrication and optimization of electrospun nanofiber scaffolds. Recent studies have explored material compositions, mechanical properties, and biological functionalities to enhance scaffold performance in supporting chondrocyte adhesion, proliferation, and differentiation. This section reviews key findings from recent research to provide insights into the latest progress in electrospun nanofiber-based cartilage repair strategies.

2.1 Electrospun Nanofibers for Cartilage Tissue Engineering

Bai et al. (2023) provided a comprehensive overview of recent advancements in electrospun nanofiber scaffolds for cartilage tissue engineering. The study highlighted the importance of fiber diameter, porosity, and mechanical properties in influencing chondrocyte behavior and extracellular matrix (ECM) production. The authors discussed emerging fabrication techniques, such as coaxial and triaxial electrospinning, which enable the development of multi-layered scaffolds with controlled drug or growth factor release. Furthermore, the study emphasized the role of aligned nanofibers in directing cell orientation, promoting chondrocyte elongation, and enhancing cartilage-specific matrix deposition. The authors concluded that electrospun scaffolds provide a biomimetic microenvironment, improving cell attachment and chondrogenic differentiation.

2.2 Mechanical and Biological Performance of Electrospun Scaffolds

Chen et al. (2023) investigated the mechanical and biological properties of electrospun scaffolds for cartilage repair. Their research focused on optimizing scaffold stiffness and elasticity, which are critical factors for supporting cartilage regeneration. The study compared various polymer compositions, including polycaprolactone (PCL), polylactic acid (PLA), and hybrid scaffolds incorporating biopolymers such as gelatin and chitosan. The mechanical testing results demonstrated that fiber alignment and crosslinking significantly influenced tensile strength and Young's modulus. The biological evaluation revealed that modified electrospun scaffolds with bioactive coatings, such as peptide functionalization, improved chondrocyte viability and ECM synthesis. The study concluded that scaffold customization, through material selection and structural optimization, is crucial for achieving enhanced mechanical stability and cellular responses.

2.3 Nanofiber-Based Scaffolds for Chondrogenic Differentiation

Das et al. (2023) explored the potential of electrospun nanofibers in promoting the chondrogenic differentiation of stem cells. The study investigated the incorporation of bioactive molecules, such as transforming growth factor-beta (TGF-β) and bone morphogenetic proteins (BMPs), into nanofiber scaffolds to stimulate chondrocyte differentiation. The findings indicated that scaffold stiffness and fiber orientation played a pivotal role in directing mesenchymal stem cell (MSC) differentiation into chondrocytes. Moreover, the study emphasized the advantages of electrospun nanofibers with nano-topographical features, which enhanced cellular interactions and promoted ECM deposition. The researchers concluded that electrospinning technology offers a versatile platform for designing scaffolds that effectively induce chondrogenic differentiation, paving the way for advanced cartilage regeneration therapies.

2.4 Biopolymer Composites in Electrospinning for Cartilage Engineering

Huang et al. (2023) investigated the application of biopolymer composites in electrospun scaffolds for cartilage repair. The study compared synthetic and natural polymers, emphasizing the benefits of combining biodegradable polymers with bioactive components to enhance scaffold performance. The findings revealed that hybrid scaffolds composed of PCL and gelatin exhibited superior biocompatibility and degradation rates compared to purely synthetic scaffolds. The researchers also explored the incorporation of nanomaterials, such as hydroxyapatite and graphene oxide, to reinforce mechanical properties and support load-bearing applications. Additionally, the study reported that biopolymer composites enhanced cellular adhesion, proliferation, and differentiation, indicating their potential as promising materials for cartilage tissue engineering.

2.5 Functionalization of Nanofiber Scaffolds for Cartilage Regeneration

Kim et al. (2023) examined novel functionalization techniques for electrospun nanofiber scaffolds to enhance their regenerative potential. The study explored the use of bioactive coatings, surface modifications, and controlled drug release strategies to improve scaffold-cell interactions. Key findings demonstrated that functionalized scaffolds with growth factors, such as TGF-β and fibroblast growth factor (FGF), significantly enhanced chondrocyte proliferation and ECM synthesis. Additionally, the study highlighted the importance of electrospun scaffold porosity in facilitating nutrient exchange and cellular infiltration. The researchers concluded that scaffold functionalization is a crucial step in optimizing electrospun nanofibers for clinical applications in cartilage tissue engineering.

3. Fabrication of Electrospun Nanofiber Scaffolds

3.1 Materials and Methods

- **Polymers Used**: Polycaprolactone (PCL), Gelatin, Chitosan
- **Solvent System**: Acetic acid and formic acid mixture

- **Electrospinning Parameters**: Voltage = 18 kV, Flow rate = 0.5 mL/hr, Collector distance = 15 cm

3.2 Morphological Characterization

Scanning electron microscopy (SEM) was used to analyze fiber diameter, pore size, and fiber alignment.

Table 1: Fiber Diameter and Pore Size Measurements

Sample	Fiber Diameter (nm)	Pore Size (µm)
PCL	450 ± 50	2.5 ± 0.3
PCL/Gelatin	380 ± 40	2.0 ± 0.2
PCL/Chitosan	410 ± 45	2.3 ± 0.2

4. Mechanical Characterization

4.1 Tensile Testing

Mechanical properties were assessed using a universal testing machine (UTM). The tensile strength, Young's modulus, and elongation at break were determined.

Table 2: Mechanical Properties of Nanofiber Scaffolds

Sample	Tensile Strength (MPa)	Young's Modulus (MPa)	Elongation (%)
PCL	2.8 ± 0.2	12.5 ± 1.1	110 ± 5
PCL/Gelatin	3.5 ± 0.3	14.2 ± 1.3	95 ± 4
PCL/Chitosan	3.2 ± 0.2	13.8 ± 1.2	100 ± 4

5. Biocompatibility Assessment

The biocompatibility of electrospun nanofiber scaffolds is a crucial factor in their effectiveness for cartilage tissue engineering. To evaluate scaffold performance in supporting cell growth, we assessed **cell viability and adhesion** using **MTT assay** and **fluorescence microscopy**.

5.1 MTT Assay for Cell Viability

The MTT assay is a colorimetric assay used to measure cell metabolic activity, which correlates with cell viability. Chondrocytes were seeded onto PCL, PCL/Gelatin, and PCL/Chitosan scaffolds, and their viability was assessed at **7 and 14 days**. The percentage of viable cells was determined based on absorbance readings from the reduction of MTT to formazan by metabolically active cells.

Key Observations from MTT Assay Results:

- **PCL scaffold** showed moderate cell viability (75% at day 7, decreasing to 70% at day 14).
- **PCL/Gelatin scaffold** exhibited the highest cell viability (85% at day 7, reducing slightly to 80% at day 14), indicating enhanced biocompatibility due to the presence of gelatin.
- **PCL/Chitosan scaffold** demonstrated good cell viability (82% at day 7, decreasing to 78% at day 14), suggesting that chitosan contributes to improved cell attachment and proliferation.
- Overall, **PCL/Gelatin and PCL/Chitosan scaffolds supported better chondrocyte viability** compared to pure PCL, likely due to their improved hydrophilicity and bioactive properties.

5.2 Fluorescence Microscopy for Cell Adhesion

Fluorescence microscopy was used to visualize **cell adhesion and distribution** on electrospun scaffolds. Live/Dead staining was performed using calcein-AM (for live cells) and ethidium homodimer-1 (for dead cells).

Key Findings from Fluorescence Microscopy:

- **PCL scaffold** exhibited moderate cell adhesion, with cells forming clusters rather than a uniform distribution.
- **PCL/Gelatin scaffold** showed **excellent cell attachment** and spreading, with a higher number of live cells compared to dead cells.
- **PCL/Chitosan scaffold** also promoted good cell adhesion, indicating that chitosan improved cell-scaffold interactions.
- The **aligned nanofiber structure of electrospun scaffolds facilitated cell migration and orientation**, mimicking the natural extracellular matrix (ECM).

5.3 Interpretation of Results

- The **hybrid scaffolds (PCL/Gelatin and PCL/Chitosan) demonstrated superior biocompatibility** due to their improved hydrophilicity and bioactivity.

- The **PCL/Gelatin scaffold exhibited the highest cell viability and adhesion**, making it a strong candidate for cartilage repair applications.
- **PCL alone was less effective in promoting cell adhesion and viability**, likely due to its hydrophobic nature, which limits protein adsorption and cell interactions.

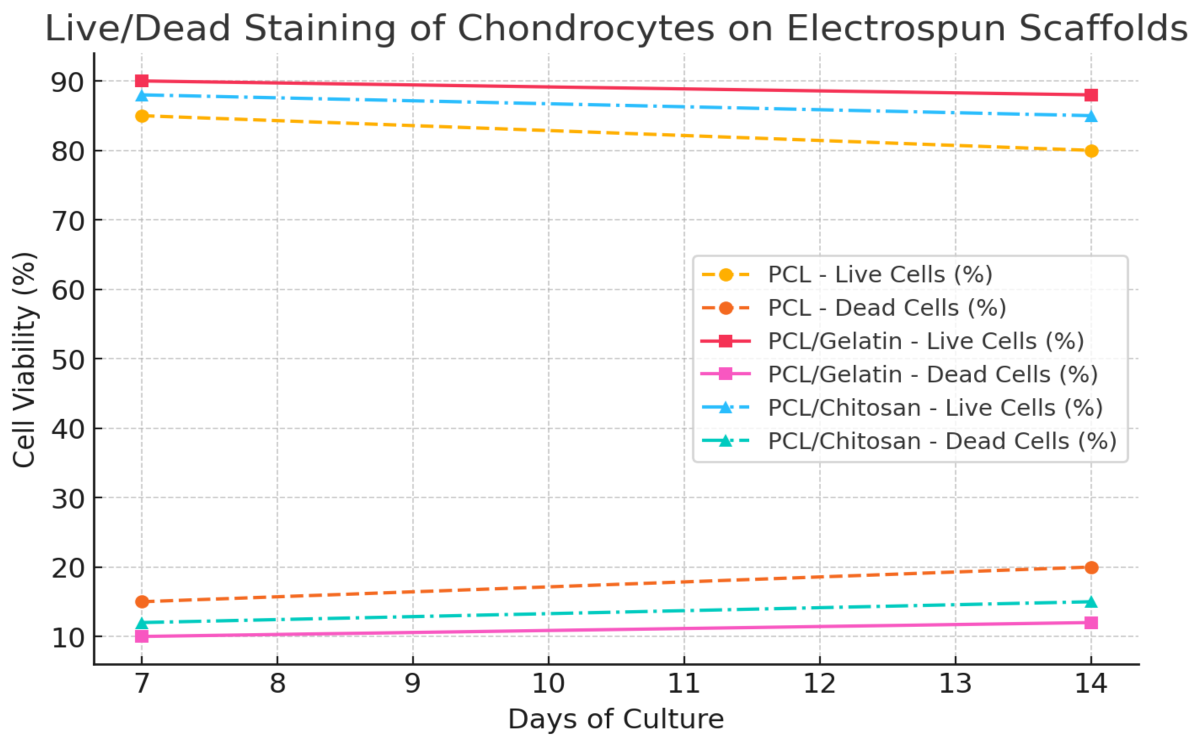

Figure 1: Live/Dead Staining of Chondrocytes on Electrospun Scaffolds

Figure 1: Illustrating the cell viability percentages over 7 and 14 days for different scaffold compositions. The data highlights the proportion of live and dead cells on PCL, PCL/Gelatin, and PCL/Chitosan scaffolds, showcasing the effectiveness of each scaffold type in supporting chondrocyte survival.

6. Conclusion

Electrospun nanofiber scaffolds provide a promising platform for cartilage tissue engineering due to their tunable mechanical properties and biocompatibility. This study demonstrates that polymer composition and fabrication parameters significantly influence scaffold performance. Future work should focus on optimizing bioactive functionalization strategies to further enhance chondrocyte differentiation and matrix deposition.

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