Research Advances in the Application of Silk Fibroin/Chitosan Composite Materials in Tissue Engineering (Postprint)

Authors: Li Dawei, He Jin, He Fengli, Liu Yali, Deng Xudong, Ye Yajing, Yin Dachuan

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Abstract

Silk fibroin (SF) and chitosan (CS) exhibit favorable biocompatibility and biodegradability; however, the various limitations of single-component SF and CS scaffold materials constrain their applications in tissue engineering research. SF/CS composite materials overcome the drawbacks of single-component SF and CS scaffolds, featuring excellent mechanical properties, good processability, tunable porosity and pore size, and complementary advantages between components. SF/CS composites fabricated through multiple methods (micro/nanoparticles, membranes, nanofibers, hydrogels, and three-dimensional porous scaffolds) have been investigated for applications in tissue engineering or repair of tissue damage in bone, cartilage, skin, nerve, adipose, cardiac, and corneal tissues. Currently, research on the application of SF/CS composite materials in tissue engineering remains in its nascent stages both domestically and internationally. This article provides a brief overview of the characteristics, preparation methods, and current research status of SF/CS composite materials in various tissue engineering applications.

Full Text

Preamble

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Li Dawei, He Jin, He Fengli, Liu Yali, Deng Xudong, Ye Yajing, Yin Dachuan**

(Key Laboratory for Space Bioscience and Biotechnology, School of Life Science, Northwestern Polytechnical University, Xi' an 710072)

**Corresponding author, email: yindc@nwpu.edu.cn

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Abstract

Silk fibroin (SF) and chitosan (CS) exhibit excellent biocompatibility and biodegradability. However, the inherent limitations of pure SF and CS scaffolds have restricted their applications in tissue engineering research. SF/CS composites overcome these shortcomings, offering superior mechanical properties, excellent plasticity, tunable porosity and pore size, and complementary advantages between components. Various SF/CS composite structures—including micro/nano particles, membranes, nanofibers, hydrogels, and three-dimensional porous scaffolds—have been investigated for tissue engineering and repair applications in bone, cartilage, skin, nerve, adipose, cardiac, and corneal tissues. Currently, research on SF/CS composites in tissue engineering remains in its early stages both domestically and internationally. This review provides a comprehensive overview of the characteristics, fabrication methods, and recent advances in the application of SF/CS composites across multiple tissue engineering domains.

Keywords: silk fibroin, chitosan, tissue engineering, tissue engineering scaffold

1 Introduction

Tissue engineering integrates principles and methods from cell biology, materials science, engineering, and clinical medicine to construct bioactive implants in vitro using scaffolds, seed cells, and growth factors, which are then implanted to repair damaged tissues or organs [1]. The tissue engineering scaffold constitutes the most fundamental element, mimicking the extracellular matrix to provide the necessary physical environment for new tissue formation. An ideal tissue engineering scaffold should be a three-dimensional porous material with good connectivity, excellent biocompatibility, defined geometry, appropriate mechanical properties, and degradation rates, and ideally should enable controlled release of growth factors [2].

Current scaffold materials primarily include medical metals (e.g., stainless steel, titanium alloys, magnesium alloys), bioceramics (e.g., calcium phosphate ceramics, bioactive glass, alumina ceramics), synthetic polymers (e.g., polycaprolactone, polylactic acid, polyethylene), and natural polymers (e.g., collagen, hyaluronic acid, chitin) [1]. As a natural polymer, silk fibroin (SF) exhibits good biocompatibility and biodegradability, and its low cost and simple preparation have enabled its use as a tissue engineering scaffold for various applications [3; 4]. However, scaffolds composed of pure SF suffer from poor mechanical properties, brittleness, difficult processing, and low water absorption. To overcome these limitations and expand its applications, researchers have combined SF with chitosan (CS) to develop SF/CS composite materials. These composites address the drawbacks of single-component SF and CS scaffolds, offering excel-

lent mechanical properties, good plasticity, tunable porosity and pore size, and complementary component advantages. Consequently, SF/CS composites have been investigated for tissue engineering and repair of bone, cartilage, skin, nerve, adipose, cardiac, and corneal tissues. Although research on SF/CS composites in tissue engineering remains in its infancy and no commercial products are currently available, significant progress has been made in understanding their properties and therapeutic efficacy.

2 Characteristics of SF/CS Composites

SF is obtained through the degumming of silk fibers, accounting for approximately 70-80% of silk fiber mass [5]. Its structure is illustrated in [Figure 1: see original paper]. SF molecules primarily contain three secondary structures: -helix (silk I), -sheet (silk II), and random coil. The -sheet (silk II) structure, formed by periodic stacking of Gly-Ala-Gly-Ala-Gly-Ser (GAGAGS) sequences, plays a crucial role in maintaining SF' s mechanical properties [6]. SF can be dissolved in high-concentration salt solutions (LiBr or CaCl) and regenerated through freeze-drying for applications in various tissue engineering contexts. Extracted from *Bombyx mori* silk, SF exhibits a high secant modulus (2.4 GPa), tensile strength (610-690 MPa), and moderate elongation (4-6%) [8]. Despite its utility, pure SF scaffolds suffer from poor mechanical properties, brittleness, difficult processing, and low water absorption.

CS, derived from deacetylated chitin, is widely sourced and cost-effective, representing the second most abundant biosynthesized polysaccharide after cellulose. CS consists of glucosamine and N-acetylglucosamine units ([Figure 2: see original paper]). Depending on its source and preparation method, CS molecular weight ranges from 300-1000 kDa with a deacetylation degree of 30-95% [9]. CS exhibits excellent biocompatibility, antimicrobial activity, cell adhesion properties, and degradability [11]. Although CS-based scaffolds have been used in bone, cartilage, and skin engineering, they suffer from poor mechanical properties, high swelling rates, and low biological responsiveness [12].

To overcome these limitations, SF and CS have been combined through blending or crosslinking to fabricate SF/CS composite scaffolds. Intermolecular hydrogen bonds, electrostatic interactions, and covalent bonds formed after crosslinking regulate the mechanical properties, swelling behavior, and aqueous stability of SF/CS composites [7]. These materials integrate the advantages of both components, yielding improved scaffold performance in several aspects: (1) **Enhanced mechanical properties**: Park et al. [13] investigated the relationship between structure and mechanical properties of SF/CS composite films, finding that at 30% CS content, crystallinity peaked and tensile strength reached 90 MPa—three times that of pure SF films and twice that of pure CS films. (2) **Improved antimicrobial activity**: Cai et al. [14] demonstrated that SF/CS composite fibers exhibit antibacterial activity against *E. coli* and *Staphylococcus aureus*. (3) **Increased water vapor and oxygen permeability**: Kweon et al. [15] evaluated SF/CS composite films for wound dressing applications,

revealing that films containing 40-50% CS exhibited excellent water vapor and oxygen permeability. (4) **Tunable properties**: Varshini et al. [16] prepared a series of SF/CS porous scaffolds with varying CS content, showing that swelling and degradation rates increased while porosity decreased with higher CS content. These improvements enable SF/CS composite scaffolds to meet diverse tissue engineering requirements.

3 Fabrication and Applications of SF/CS Composites

Various methods and processes can produce SF/CS composites with different structural morphologies: (1) **Emulsion-diffusion and spray-drying** produce SF/CS nanoparticles primarily for mechanical reinforcement and as drug/growth factor carriers [17-19]. (2) **Solution casting** yields SF/CS membranes for wound healing and membrane-based tissue engineering such as skin and cornea [20; 21]. (3) **Electrospinning** generates SF/CS nanofibers for vascular, neural, and bone defect repair [22; 23]. (4) **Crosslinking** creates SF/CS hydrogels for drug delivery and three-dimensional cell culture [24; 25]. (5) **Three-dimensional porous SF/CS scaffolds** are primarily fabricated via particle leaching, freeze-drying, and 3D printing, with their respective advantages and disadvantages summarized in . These 3D scaffolds have been widely used in bone, cartilage, and adipose tissue engineering [26-28].

Comparison of fabrication methods for SF/CS 3D porous scaffolds

Method	Advantages	Disadvantages
Particle Leaching	Simple operation, low cost, controllable pore size and porosity	Poor internal connectivity between pores, unsuitable for scaffolds thicker than 2 mm, residual organic solvents difficult to remove completely
Freeze-drying	Simple operation, no organic solvents, high porosity, good internal pore connectivity	Long preparation time, difficult to fabricate hierarchical structures, small pore size
3D Printing	Can fabricate complex shapes, high precision, tunable pore size/porosity, controllable pore geometry	Requires specialized equipment, residual organic solvents difficult to remove during fabrication

4 Applications in Specific Tissue Engineering Fields

4.1 Bone Tissue Engineering

Bone defect repair and reconstruction represent a major focus in tissue engineering, with SF/CS composites finding extensive applications. Natural bone is a connective tissue composed of collagen (COL), hydroxyapatite (HAP), and calcified extracellular matrix, requiring scaffolds with appropriate toughness and matrix deposition capacity [1]. Zeng et al. [29] prepared SF/CS scaffolds via freeze-drying and, through comprehensive analysis of porosity, pore size, swelling, water absorption, and degradation rates, determined that an SF:CS ratio of 4:6 optimally promoted early adhesion and proliferation of osteosarcoma MG63 cells while stimulating extracellular matrix secretion by osteoblasts.

SF/CS/HAP three-phase composite scaffolds are frequently used for osteoblast culture and bone defect repair. Qi et al. [30] combined particle leaching and freeze-drying to disperse nano-hydroxyapatite (nHAP) within SF/CS scaffolds, demonstrating significantly enhanced mechanical properties compared to SF/CS scaffolds alone, with MG63 cells showing improved adhesion and proliferation. Lai et al. [31] incorporated nHAP into SF/CS solutions to fabricate SF/CS/nHAP nanofiber scaffolds via electrospinning, revealing that human mesenchymal stem cell (hMSC) differentiation correlated with nHAP content, with higher nHAP promoting greater differentiation. In vivo implantation demonstrated bone repair potential. SF/CS/HAP hydrogels prepared via in situ deposition supported proliferation and differentiation of pre-osteoblast MC3T3-E1 cells [32]. SF/CS/HAP membranes fabricated using thermally induced phase separation showed comparable bone volume and density to collagen membranes in rat calvarial defect models [33].

Growth factor modification effectively regulates bone cell growth and differentiation to promote defect repair. Tong et al. [34] loaded vascular endothelial growth factor (VEGF) onto SF/CS scaffolds, demonstrating enhanced growth and proliferation of human embryonic osteoblasts. Shalumon et al. [35] incorporated bone morphogenetic protein-2 (BMP-2) into SF/CS/HAP nanofiber membranes, showing that BMP-2 release maintained osteoinductive activity and promoted osteogenic differentiation of hMSCs. Implantation of hMSC-seeded SF/CS/HAP/BMP-2 scaffolds in nude mouse dorsal bone defect models confirmed ectopic bone formation through immunohistochemistry and histological staining.

4.2 Cartilage Tissue Engineering

Cartilage is an avascular connective tissue composed of extracellular matrix and chondrocytes [1]. Collagen type II (COL-2) and glycosaminoglycans (GAGs) are major extracellular matrix components that critically regulate cartilage formation both in vitro and in vivo. CS structure resembles GAGs and hyaluronic acid in cartilage, making it widely used for cartilage repair and reconstruction.

Bhardwaj et al. [36] compared the chondrogenic differentiation capacity of MSCs on two single-component SF scaffolds (from *Bombyx mori* and *Antheraea mylitta*) versus SF/CS scaffolds, demonstrating superior chondrogenic differentiation on SF/CS scaffolds. They further reported that bovine chondrocytes secreted maximal GAGs and COL-2 at an SF:CS ratio of 1:1 [37]. In rabbit knee joint injury models, MSC-seeded SF/CS scaffolds showed superior repair capacity compared to cell-free scaffolds [38]. Zang et al. [39] demonstrated that perichondrium-wrapped SF/CS scaffolds significantly improved cartilage formation in vivo. CS can also be incorporated as nanoparticles to modulate chondrocyte growth. Naeimi et al. [28] embedded CS nanoparticles into silk fibroin/chondroitin/alginate scaffolds, enhancing mechanical properties and increasing expression of GAGs, COL-2, and SOX9 genes in adipose-derived stem cells (ASCs).

4.3 Skin Tissue Engineering

Skin, the body' s largest organ, serves as an interface protecting underlying tissues from pathogens and microorganisms [1; 2; 40]. Skin defect repair represents a significant clinical challenge. Ideal skin tissue engineering scaffolds should cover wounds completely, elicit no immune response, enhance wound healing, reduce patient discomfort, and minimize scar formation [41].

Witoo et al. [42] cultured fibroblasts on SF/CS membranes, demonstrating not only absence of cytotoxicity but also enhanced fibroblast proliferation and COL-1 expression. Guang et al. [43] found that SF/CS membranes supported skin regeneration in rat wound models. Gu et al. [44] improved SF/CS membrane stability through alginate dialdehyde crosslinking, achieving water absorption and vapor permeability suitable for wound dressings while promoting cell adhesion and proliferation. Additional natural polymers can be combined with SF and CS to enhance scaffold performance. Sharma et al. [45] fabricated quaternary composite scaffolds from silk fibroin, chitosan, alginate, and gelatin via foaming, demonstrating excellent cell viability for mouse fibroblasts (L929). Zhou et al. [46] incorporated SF nanoparticles into N-carboxyethyl chitosan/poly(vinyl alcohol) electrospun scaffolds, improving mechanical properties and biocompatibility for skin repair applications. Li et al. [47] prepared SF/CS scaffolds containing bioactive glass via freeze-drying, showing not only good biocompatibility and tissue repair capacity but also enhanced vascularization during wound healing.

4.4 Neural, Adipose, Cardiac, and Corneal Tissue Engineering

The nervous system comprises a complex biological network of peripheral and central nerves [40]. While minor injuries can self-repair, tissue engineering approaches are crucial for larger defects requiring surgical intervention. Wei et al. [48] seeded ASCs onto SF/CS scaffolds for rat sciatic nerve defect repair, demonstrating significantly improved nerve continuity and functional recovery based on gait analysis and histology. Yao et al. [49] combined bone marrow

mononuclear cells (BM-MNCs) with SF/CS scaffolds for rat sciatic nerve repair, achieving regeneration comparable to autologous nerve grafts.

Adipose tissue, composed of aggregated adipocytes, influences various physiological activities including insulin sensitivity, blood pressure, and inflammatory responses [1]. Kang et al. [26] seeded ASCs onto CS-modified SF scaffolds, observing robust cell adhesion and proliferation with abundant adipocyte formation after 14 days of adipogenic induction. They further investigated adipose tissue formation using lentiviral vectors carrying the VEGF-165 gene to infect human adipose-derived stem cells on CS-modified SF scaffolds, demonstrating that VEGF-165 lentivirus did not impair adipogenic differentiation while providing sustained VEGF-165 delivery [50].

SF/CS scaffolds have also been investigated for cardiac and corneal repair. Chi et al. [51] implanted chitosan/hyaluronic acid/silk fibroin scaffolds in rat myocardial infarction models, showing significant reductions in left ventricular diameter, increased wall thickness, and improved fractional shortening after 8 weeks. These scaffolds also enhanced VEGF secretion, demonstrating potential for cardiac repair. Guan et al. [52; 53] conducted extensive studies on SF/CS scaffolds for corneal transplantation. They first implanted acellular SF/CS membranes into rabbit corneal stroma to evaluate biocompatibility, then co-cultured rabbit corneal epithelial and stromal cells on SF/CS membranes before implantation into corneal defect models. The reconstructed lamellar cornea resembled native tissue, with high expression of K3/12 genes in epithelial cells and proteins in stromal cells, and no immune response after 12 weeks.

5 Summary and Outlook

As natural polymers with excellent biocompatibility and degradability, SF/CS composites hold broad promise in tissue engineering. They can be further combined with HAP, COL, hyaluronic acid, and other materials to fabricate scaffolds with tailored properties for diverse applications. Despite extensive research, several challenges remain, including inadequate mechanical properties for load-bearing bone applications and mismatched degradation rates relative to tissue formation kinetics.

Future research should focus on: (1) Developing novel SF/CS scaffold fabrication technologies and optimizing processing parameters to create scaffolds meeting diverse tissue engineering requirements; (2) Enhancing cell adhesion, proliferation, and differentiation through surface modification to provide experimental guidance and theoretical foundations for engineered tissues and organs; and (3) Addressing limitations in drug loading efficiency and sustained release capabilities of SF/CS scaffolds for drug delivery applications.

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Note: Figure translations are in progress. See original paper for figures.