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Application of silk fibroin composite scaffold in bone tissue engineering

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Abstract: *As a natural protein, silk protein has superior biocompatibility, biodegradability and mechanical properties, and can be used in different forms for bone tissue repair in bone tissue engineering as a good biomaterial. The treatment of bone defect has always been a serious challenge for orthopedic surgeons. However, the traditional treatment of bone defect is autologous bone and bone allograft, with limited source and secondary injury with sampling site; the allograft faces ethical problems, rejection, and viral transmission risk. Tissue engineering is an important means to solve tissue and organ defects. It establishes a three-dimensional space complex composed of cells and biological materials and fills the defects, so as to repair the effect Bone tissue scaffold with filim protein plays a huge role in orthopedics, and is expected to bring good news to more patients with bone diseases in the future. In this paper, we review the structural characteristics, biological properties and applications of filamentous proteins in bone tissue engineering.*

Keywords: *silk protein, scaffolding, osteogenesis, bioengineering*

1. Introduction

Maxillofacial fractures, trauma, and tumors often result in bone defects of varying degrees in the maxillofacial region, which seriously affect the speech and masticatory functions, facial aesthetics, and psychological health of patients [1]. Currently, the main clinical methods for treating bone defects are autologous bone grafting, allogeneic bone grafting, and bone replacement material implantation repair. Among them, autologous bone grafting may cause complications for the patients themselves, secondary wounds, and slower healing; allogeneic bone grafting may reduce the pain of patients, but the immune rejection after transplantation may aggravate the condition and increase the cost of surgery [2]. Bone replacement materials can provide a stable environment for the hard bone defect site, giving good mechanical support to the injured area, which in turn promotes healing [3]. It is for these reasons that tissue engineering (TE) repair of bone defects has attracted widespread interest.

Scaffold materials in bone tissue engineering have an important role in the study of scaffold materials, which provide a suitable growth environment and mechanical support for cells during bone tissue regeneration[4] . Silk has been commercially available in the traditional textile industry for more than 4000 years and has been approved by the US Food and Drug Administration (FDA) for use in sutures due to its outstanding physical properties such as luster, lightness, flexibility and strong mechanical strength and has been used in biomedical applications for the past 2 years[5] . Sericin protein (SF) derived from silk is a unique natural high-fiber protein with many desired physicochemical properties such as excellent biocompatibility, biodegradability, bioresorbability, low immunogenicity, and tunable mechanical properties and thus has been used as a potential natural biopolymer for bone tissue engineering [6]. In this paper, we review the biological structural characteristics of SF, the tissue engineering elements of SF composite scaffolds, and the progress in the application of SF composite scaffolds.

2. Structural characteristics of SF

Silk protein is produced by various insects such as silkworms, spiders, lace, fireflies, flies and mites, and silk from silkworms and spiders is the most commonly used for biological applications. The most commonly used silk originates from Bombyx mori, a silkworm that feeds on mulberry silkworms and produces higher quality fibers than most non-mulberry families [7]. Silkworm silk contains core structural

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SF proteins (70-80%) and colloidal silk gliadin (20-30%). Colloidal silk gliadin is wrapped around the silk protein and is usually soluble and can be removed by a debinding process.The main components of SF are proteins, small amounts of lipids and polysaccharides.SF has a large molecular weight modular hydrophobic structure interrupted by smaller hydrophilic groups.SF contains two main chains: a hydrophobic heavy (H-) chain and a hydrophilic light (L-) chain. The H-chain, L-chain and P25 are assembled in a 6:6:1 molar ratio to form silk [8]. The silk protein chain molecule contains a variety of amino acids, including glycine (Gly, G), alanine (Ala, A), serine (Ser, S), etc. The main structural element is the repetitive GAGAGS sequence. Hydrophobic structural domains composed of repetitive amino acid sequences are assembled into nanocrystals (β-fold), while hydrophilic connections between these hydrophobic structural domains form amorphous structural domains of secondary structure. The elasticity of the silk is determined by the chain conformation in the amorphous block [9]. Fundamentally, the SF structure consists mainly of a group of crystalline and amorphous regions, with amorphous region I and crystalline region II being the main crystal structures of SF, where amorphous region I (Silk I) is a substable crystal structure including bound water molecules, while crystalline region II (Silk II) is the most stable state because of the tight hydrogen bonding links between neighboring peptides, leading to increased mechanical properties, rigidity and tensile strength. Silk I and silk II conformations can be transformed by changing conditions such as temperature, stress, solvent polarity, and pH. Their mechanical behavior can be controlled by precise manipulation of the crystalline and amorphous domains at the nanoscale [10].

3. Biological performance characteristics of SF composite stents

3.1 Biocompatibility of SF composite scaffolds

Biocompatibility refers to the physical, chemical, and biological reactions that result from the interaction between a material and an organism. In general, the degree of compatibility of a material with the body after implantation and whether it causes toxic effects on human tissues. Biocompatibility is a key factor for successful implementation of scaffolds, which enables cells to adhere to the scaffold surface and migrate into the scaffold to proliferate and differentiate. The SF composite scaffold was found to be more biocompatible when used in bone tissue engineering, and Jo et al. conducted an in vivo trial to evaluate the effectiveness of alginate/HAP/SF composite as a bone substitute, showing no infection and reduced immunogenicity for up to four weeks. Tumor necrosis factor-α (TNF-α) expression levels were significantly reduced, while Runx2 and fibroblast growth factor (FGF)-23 were expressed at higher rates in the tumor necrosis factor[11].Li et al. induced hydrogel formation from degummed filamentous protein (SF) by dimyristoylphosphatidylglycerol (DMPG), using sheep iliac satellite cells as seed cells, at different DMPG The absorbance of the SF scaffold was tested by enzyme standardization at different concentrations of DMPG, and it was found that all SF scaffold groups showed no decrease in cell viability compared to the blank group, indicating that the SF scaffold was not cytotoxic and that the DMPG-induced SF scaffold group had excellent biocompatibility and biosafety, as well as a promotion effect on cell proliferation[12].Reza Eivazzadeh-Keihan et al. prepared a carboxymethyl/filin/hydrogen cellulose/silk proteins/magnesium hydroxide nanocomposite scaffolds, and measured the biological properties of CMC hydrogel/SF/Mg(OH)2 nanocomposite scaffolds in vitro and in vivo, and observed excellent hemocompatibility and high antibacterial activity of SF scaffolds, and the results of in vivo wound healing assay found that SF nanocomposite scaffold-treated mice had faster wound healing rate than the control group within 8 days [13].

3.2 Biodegradability of SF composite stents

Different tissue regeneration cycles vary and the degradation rate should match the rate of new tissue formation and it is critical to adjust this rate. Morphological parameters (e.g., porosity, pore size, thickness, surface area-volume ratio, etc.) have a significant effect on the degradation properties of SF materials.Luo et al. related the initial pore size to the biodegradation of scaffolds. By controlling the concentration of SF solution, scaffolds with obvious pore morphology were prepared, and the higher the concentration, the smaller the pore size and the slower the degradation rate [14]. Zhang et al. prepared SF scaffolds with adjustable degradation rate by controlling dissolution, hydrolysis and freeze-drying, and found that the degradation behavior of SF scaffolds could be well regulated in in vitro enzymatic degradation and in vivo subcutaneous implantation real. Immunohistochemical staining experiments showed that SF scaffold degradation products could promote endothelial cell proliferation [15].

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3.3 Biechanechanproperties of the SF composite scaffold

The stent should adapt to the mechanical properties of natural bone and conduct appropriate loads. SF without silk glue exhibits better mechanical properties and helps control long-term conformation and stability. The modulus can reach 5-12 GPa for filament-containing glues and 15-17 GPa without filaments.This study illustrates that SF has great shear strength, tensile strength, and fracture resistance, making it an ideal material for bone construction [16]. SF composite scaffolds have better mechanical and biological properties compared to monophasic SF scaffolds.Tian's people prepared porous composite scaffolds made of SF/WK (wool keratin) with appropriate concentration and mass ratio using freeze-drying technique. The results showed that the composite scaffolds were insoluble in water and had good mechanical properties with a porosity above 80% and a pore size above 200 μm. Compared with pure SF scaffolds, WK's increased the pore size and connectivity of SF composite scaffolds. The heat resistance and water solubility of WK enhanced the thermal and mechanical properties of the composite scaffolds [17]. zhao et al. used 3D printing technology to fabricate SF mixed with polyvinyl alcohol (PVA)/nHA and found that compared to PVA/nHA composite scaffolds, SF/PVA/nHA scaffolds possessed better porosity, compressive force properties and regular continuous scaffold structure, which provided an experimental basis for 3D printing SF composite scaffolds [18].

4. Application of the SF composite scaffold

Bone is a special connective tissue composed of 35% organic fraction and 60% inorganic matrix. sF has good biological and mechanical properties and thus is widely prepared as tissue engineering composites [19].

4.1 Application of the fibrofibroin composite scaffold for osteogenesis in bone tissue engineering

Repair of large segmental bone defects is the focus of bone tissue engineering, and a single SF scaffold has a limited repair capacity in the repair of a large number of bone defects. To increase the repair capacity of SF materials, bioactive factors and osteoblasts are often added to SF to prepare a composite scaffold. Hydroxyapatite is (HA) is the main component of the inorganic mineral phase of bone, Peng et al. prepared nanoscale hydroxyapatite (nHA) and SF into composite scaffolds to induce defect formation in rabbit radius, and after implantation of the scaffolds, X-ray scans, specimen observation and histopathological examination were performed, and SF/nHa scaffolds were observed to have good osteogenic capacity [20]. Cells of dental tissue origin can be easily obtained from extracted teeth, and studies have demonstrated that dental stem cells are an effective source for manufacturing skeletal structures. Dental pulp stem cells (DPSC) isolated from dental pulp tissue can differentiate into osteoblast precursors under stimulated conditions [21].Jin et al. inoculated DPSC in culture into SF /nHa composite scaffolds and implanted them into prepared rabbit cranial defects.The SF composite scaffolds occupied the defect space, facilitated new bone formation, and exhibited good structural maintenance at all loci. The scaffold was covered by an overlying periosteal surface and the silk fibers were surrounded by connective tissue of extracellular matrix. inflammatory cells were no longer present around the fibrous network after 4 weeks, and early new bone formation was observed to occur mainly at the periphery of the defect, and the SF/nHa composite scaffold inoculated with DPSC had significant osteogenic capacity compared with the control group [22]. 3D printing technology to construct tissue-engineered bone allows for extreme bone defect. For personalized treatment, Liu et al. prepared bone marrow mesenchymal stem cell membranes compounded with 3D printed horse deer antler powder/SF /PVA scaffolds for repair and implanted into the extreme defect of sheep jaws, and a large amount of new bone was formed after 3 months. Histology revealed that the mRNA expression levels of bone bridging protein, osteocalcin, and type I collagen were significantly higher than those of the control group [23].

4.2 Application of vascularization in bone tissue engineering of a fibroin protein composite scaffold

The regeneration of blood vessels is essential for the regeneration of bone tissue. Vascular endothelial growth factor (VEGF) not only promotes osteoblast differentiation, but also causes neovascularization. It was found that embedding VEGF in RSF-calcium phosphate-poly (lactic-co-glycolic acid) scaffolds maintained approximately 28% bioactivity after up to 83 days of VEGF release in vitro [25]. Another study performed in vivo found that SF/nHa scaffolds in which low doses of osteoinductive and angiogenic factors, bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF), were embedded and released in a controlled manner, were implanted in rabbit cranial defects

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and found that the rapid initial release of VEGF mimicked its expression in the early stages of bone healing and promoted angiogenesis, and that BMP-2 with VEGF can lead to a synergistic effect on vascularized bone regeneration [26].Guo et al. used two methods to prepare SF scaffolds - random SF scaffolds (RSS) and aligned SF scaffolds (ASS) - and their effects on angiogenesis and wound healing in rats. In vivo results showed that both types of dressings promoted wound epithelialization. Both composite scaffolds significantly accelerated wound healing compared to the control (untreated) group for both dressing types, suggesting that SF composite scaffolds promote wound neovascularization, which is essential for effective wound healing.

5. Conclusions

Silk protein derived from silkworm cocoons is an FDA-approved biomaterial that has been widely accepted for TE applications due to its unique biomedical properties, mechanical properties, and tunability. SF has been made into various forms, including films, mats, artificial fibers, sponges, and hydrogels, which have been successfully used in various TE applications. While 3D printing biotechnology is gradually becoming the focus of research in bone tissue engineering, combining SF with 3D bioprinting technology will have a wide research future.

References

[1] Zhao J, Zhou YH, Zhao YQ, et al. Oral cavity-derived stem cells and preclinical models of jaw-bone defects for bone tissue engineering. [J]. Stem Cell Res Ther. 2023; 14(1):39.
[2] Lim HK, Choi YJ, Choi WC, Song IS, Lee UL. Reconstruction of maxillofacial bone defects using patient-specific long-lasting titanium implants. [J]. Sci Rep. 2022; 12(1):7538.
[3] Ferguson BM, Entezari A, Fang J, Li Q. Optimal placement of fixation system for scaffold-based mandibular reconstruction. [J]. Mech Behav Biomed Mater. 2022; 126:104855.
[4] Chocholata P, Kulda V, Babuska V. Fabrication of Scaffolds for Bone-Tissue Regeneration. [J]. Materials (Basel). 2019; 12(4):568.
[5] Huang W. Ling S. Li C. Omenetto FG. Kaplan DL . Silkworm silk-based materials and devices generated using bio-nanotechnology. [J]. Chem Soc Rev. 2018; 47(17):6486-6504.
[6] Sangkert S, Juncheed K, Meesane J. Osteoconductive Silk Fibroin Binders for Bone Repair in Alveolar Cleft Palate: Fabrication, Structure, Properties, and In Vitro Testing. [J]. Funct Biomater. 2022; 13(2):80.
[7] Fang G. Sapru S. Behera S. et al. Exploration of the tight structural-mechanical relationship in mulberry and non-mulberry silkworm silks. [J]. Mater Chem B. 2016; 4(24):4337-4347.
[8] Asakura T. Structure of Silk I (Bombyx mori Silk Fibroin before Spinning) -Type II β-Turn, Not α-Helix. [J]. Molecules. 2021; 26(12):3706.
[9] McGill M, Holland GP, Kaplan DL. Experimental Methods for Characterizing the Secondary Structure and Thermal Properties of Silk Proteins. [J]. Macromol Rapid Commun. 2019; 40(1):e1800390.
[10] Wang HY, Zhang YQ, Wei ZG. Dissolution and processing of silk fibroin for materials science. [J]. Crit Rev Biotechnol. 2021; 41(3):406-424.
[11] Jo YY, Kim SG, Kwon KJ, et al. Silk Fibroin-Alginate-Hydroxyapatite Composite Particles in Bone Tissue Engineering Applications in Vivo. [J]. Int J Mol Sci. 2017; 18(4):858.
[12] Yi BC, Zhang HL, Yu ZP, Yuan HH, Wang XL, Shen YB, Bao JY, Lou XX, Zhang YZ. Osteoinductivity and performance of silk fibroin solution. [J]. Zhongguo Zuzhi Gongcheng Yanjiu. 2016; 20(52):7788-7795
[13] Eivazzadeh-Keihan R, Khalili F, Khosropour N, et al. Hybrid Bionanocomposite Containing Magnesium Hydroxide Nanoparticles Embedded in a Carboxymethyl Cellulose Hydrogel Plus Silk Fibroin as a Scaffold for Wound Dressing Applications. [J]. ACS Appl Mater Interfaces. 2021; 13(29): 33840-33849.
[14] Gil ES, Park SH, Hu X, Cebe P, Kaplan DL. Impact of sterilization on the enzymatic degradation and mechanical properties of silk biomaterials. [J]. Macromol Biosci. 2014; 14(2):257-269.
[15] Zhang L, Liu X, Li G, Wang P, Yang Y. Tailoring degradation rates of silk fibroin scaffolds for tissue engineering. [J]. Biomed Mater Res A. 2019; 107(1):104-113.
[16] Kwak HW, Ju JE, Shin M, Holland C, Lee KH. Sericin Promotes Fibroin Silk I Stabilization Across a Phase-Separation. [J]. Biomacromolecules. 2017; 18(8):2343-2349.
[17] Tian Y, Wu Q, Li F, et al. A flexible and biocompatible bombyx mori silk fibroin/wool keratin composite scaffold with interconnective porous structure. [J]. Colloids Surf B Biointerfaces. 2021; 208: 112080.

ISSN 2706-6819 Vol.5, Issue 6: 18-22, DOI: 10.25236/IJFM.2023.050604

[18] Ding Liuchuang, Zhao Xiaoqi, Han Xiangzhen, Zhou Qiqi, et al. Performance comparison of three-dimensional printed silk fibroin/polyvinyl alcohol /nano-hydroxyapatite scaffold and polyvinyl alcohol /nano-hydroxyapatite scaffold. [J]. Stomat0ology. 2018; 07-0598-05.
[19] Sun W, Gregory DA, Tomeh MA, Zhao X. Silk Fibroin as a Functional Biomaterial for Tissue Engineering. [J]. Int J Mol Sci. 2021; 22(3):1499.
[20] Wang L, Nan X, Hou J, et al. Preparation and biological properties of silk fibroin/nano-hydroxyapatite/hyaluronic acid composite scaffold. [J]. Biomed Mater. 2021; 16(4).
[21] Kim BC, Bae H, Kwon IK, et al. Osteoblastic/cementoblastic and neural differentiation of dental stem cells and their applications to tissue engineering and regenerative medicine. [J]. Tissue Eng Part B Rev. 2012; 18(3):235-244.
[22] Park JY, Yang C, Jung IH, et al. Regeneration of rabbit calvarial defects using cells-implanted nano-hydroxyapatite coated silk scaffolds. [J]. Biomater Res. 2015; 19:7.
[23] Liu Xy, Li L, Zhang K, Li J, Han Xz, He Hy. Osteogenesis using bone marrow mesenchymal stem cell sheets combined with threedimensional printed Cervus elaphus antler powder/silk fibroin/ polyvinyl alcohol scaffold in vivo. [J]. Zhongguo Zuzhi Gongcheng Yanjiu. 2021; 25(34):5420-5426.
[24] Farokhi M, Mottaghitalab F, Shokrgozar MA, Ai J, Hadjati J, Azami M. Bio-hybrid silk fibroin/calcium phosphate/PLGA nanocomposite scaffold to control the delivery of vascular endothelial growth factor. [J]. Mater Sci Eng C Mater Biol Appl. 2014; 35:401-410.
[25] Wang Q. Zhang Y. Li B. Chen L. Controlled dual delivery of low doses of BMP-2 and VEGF in a silk fibroin-nanohydroxyapatite scaffold for vascularized bone regeneration. [J]. Mater Chem B. 2017; 5(33): 6963-6972.
[26] Guo P, Du P, Zhao P, et al. Regulating the mechanics of silk fibroin scaffolds promotes wound vascularization. [J]. Biochem Biophys Res Commun. 2021; 574:78-84.