Application of silk fibroin in biomedical engineering

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Abstract: Silk fibroin is the main component of silk and is known for its excellent biocompatibility, degradability, mechanical properties, and safety. It is non-toxic, and its raw materials are readily available, making it a popular material in the preparation and research of biomedical materials. This article reviews the healthcare benefits of silk fibroin, such as its role in lowering blood sugar and inhibiting bacterial growth, as well as its applications and development prospects in biomedical engineering fields, including drug release, hemostasis, wound healing, tissue engineering scaffolds, and other biomedical materials.

Keywords: Silk fibroin, Biomedicine, Biomedical engineering, Application

1. Introduction

Silk protein is mainly composed of two parts: silk fibroin (Silk Fibroin, SF) and sericin. The silk fibroin accounts for about 70%–80%of the total mass of silk protein and is the main component of silk protein. It is composed of 18 amino acids, including glycine, alanine, and serine, connected by multiple peptide bonds. For a long time, the use of silk has been limited to high-end textile fibers. However, due to its excellent biocompatibility and degradability, and its non-toxic, pollution-free, and non-irritating nature, the degradation rate of silk fibroin can be regulated by preparing it into different morphological structures. In recent years, it has been widely applied in the fields of biology and medical engineering.

2. Applications of silk fibroin in healthcare

2.1. 2.1 Blood glucose lowering effects

The use of boiled silkworm cocoons for the treatment of diabetes has been documented in ancient China. In recent years, researchers have achieved promising results in diabetes treatment by feeding animals or treating cells with hydrolysates of silk fibroin. Hyun et al. [3] demonstrated that silk fibroin hydrolysates, when applied to the 3T3-L1 cell line, could accelerate glucose metabolism and glycogen turnover independent of insulin. Additionally, silk fibroin regulates glucose transporter 1(GLUT1), increasing its expression on the cell surface and enhancing GLUT4 translocation, suggesting that the high blood glucose symptoms in diabetic patients may be improved under the influence of silk fibroin. Furthermore, Jun-Hong Park et al. [4] found that HIT-T15 pancreatic β-cells, which are prone to cell death induced by high glucose concentrations, showed reduced cell death after treatment with 50 mg/mL silk fibroin. The silk fibroin treatment also lowered cellular reactive oxygen species (ROS)levels and increased the immunoreactivity of proliferating cell nuclear antigen (PCNA). TU NEL assays revealed that silk fibroin protected HIT-T15 cells from glucose-induced apoptosis, indicating that silk fibroin may protect cells from death by reducing ROS levels. As a potential therapeutic agent for diabetes or hyperglycemia, silk fibroin can not only lower blood glucose levels and alleviate symptoms of diabetes and its complications but also avoid adverse reactions associated with some diabetic medications, making it a promising natural material in the biomedical field [5].

2.2. Antibacterial effects

When silk fibroin is hydrolyzed into silk fibroin peptides and placed in a natural environment for several hours, a reduction in the number of bacterial colonies compared to the control group can be observed, indicating that silk fibroin peptides possess certain antibacterial properties[6]. When silk fibroin hydrolysate

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is added to spicy kimchi, it is found to significantly inhibit the growth of lactic acid bacteria. Based on its favorable antibacterial characteristics, corresponding antibacterial drugs can be developed for utilization [7].

Table 1 Comparison of the Inhibitory Effects of Silk Fibroin Peptides at Different Concentrations on Mixed Bacteria in the Air (Colony Counts)

Placement Time/h	1%Silk Fibroin Peptides	3%Silk Fibroin Peptides	5%Silk Fibroin Peptides	10%Silk Fibroin Peptides	Control
1	5	2	0	0	7
2	6	1	1	0	16
3	17	9	5	3	18
4	15	17	5	4	42
5	39	32	15	3	55

3. Applications of silk fibroin in biomedical engineering

3.1. Drug release

Due to its slow biodegradability, non-toxicity to biological organisms, excellent biocompatibility, unique mechanical properties, and distinctive amino acid composition, silk fibroin can be chemically modified on its surface through the amino groups or side chains of certain amino acids. With the development and trends in drug release biomaterials, silk fibroin has become a popular research subject as a carrier for controlled drug release. As a drug release carrier, silk fibroin can reduce the burst release of drugs when they enter the body, effectively slow down the release rate of drugs absorbed through the skin, stabilize the drug's action within the body, minimize drug waste, and inhibit the evaporation loss of certain volatile components, thereby significantly enhancing drug efficacy.

Currently, silk fibroin is mainly used in several physical forms as a controlled-release carrier material, including hydrogels, film coatings, microparticles, nanoparticles, channels, scaffolds, and tablets. These forms can be tailored to meet the requirements of drug molecular weight and release rate, allowing for the production of various specifications of drug carriers [8-9]. For example, Lerdchai K et al.in Thailand [10] encapsulated the anticancer compounds curcumin and DHA in a silk fibroin/gelatin (SF/G) sponge as a drug release system for cervical cancer treatment. The SF/G sponge is non-toxic to normal cells and controls the slow release of curcumin and DHA, thereby enhancing the inhibitory effect on cancer cell growth.

3.2. Hemostatic function

Commonly used hemostatic materials in current medical practice mainly include hemostatic gauze, hemostatic bandages, and hemostatic fibers. These materials have several drawbacks during use, such as longer hemostasis time, higher risk of causing allergies, tendency to adhere to wounds, and lower efficacy in preventing wound infections. Due to its non-toxicity and low inflammatory response, silk fibroin has been the subject of research for the development of new hemostatic materials in recent years. Xu Yayan et al. [11] used a Ca Cl_2-ethanol-water ternary system to hydrolyze silk fibroin at a temperature of 48°C, obtaining silk fibroin peptides with an average molecular weight of 30 k D. Experiments conducted on a rat liver hemostasis model revealed that rats treated with 30 k D silk fibroin peptides had less blood loss and shorter hemostasis time. The hemostatic effect was comparable to that of the clinically used hemostatic powder Arista. Additionally, silk fibroin peptides exhibited better antibacterial properties and were non-cytotoxic, indicating a broad prospect for the development of rapid hemostatic materials.

3.3. Wound healing

Silk fibroin has the ability to promote the regeneration of skin tissue and accelerate wound healing. Skin wounds treated with silk fibroin heal more rapidly than those treated with saline [12]. The occurrence of trauma often leads to microbial infections, making it essential for wound dressings to have good sealing

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properties, antibacterial activity, and moisture retention to facilitate wound healing. With its breathability, strong moisture retention, ease of shaping, and non-toxicity, silk fibroin is an ideal candidate material for preparing skin wound dressings[13-15].Currently, various types of silk fibroin wound dressings have been developed, including silk fibroin films[15-16],silk fibroin gels[17],silk fibroin sponges[13,18],silk fibroin nanofibers, and silk fibroin wound dressings loaded with growth factors[22]or plant active ingredients [23]. These dressings can meet the diverse needs of wound treatment[24].

3.4. Tissue Engineering Scaffolds

In recent years, with the increasing research focus on silk fibroin in the biomedical field, a variety of tissue engineering products related to vascular, cartilage, and bone tissue repair, as well as skin and nerve tissue regeneration, have been developed. These materials provide excellent scaffolds for cell growth and tissue repair [25-28].

3.4.1. Artificial Blood Vessels

Silk fibroin can promote cell proliferation and has good tissue compatibility and mechanical properties, as well as a slow degradation rate, making it a candidate material for the development of artificial blood vessels [25]. Zhu Chen hui et al. [29] mixed human-like collagen with silk fibroin at a mass ratio of 7:3 to create a human-like collagen–silk fibroin tubular scaffold. This scaffold has a uniform porous structure with pore sizes of (60±5) μ m and a porosity of over 85%; it exhibits ideal mechanical properties, with a strain of 50%±5%and a stress of (332±16) kPa. Its slow degradation rate enhances cell adhesion and proliferation, and it has good biocompatibility. Chen Jie et al. used soft lithography to prepare four types of silk fibroin membranes with different microstructures, and investigated their effects on the growth, morphology, arrangement, and proliferation of human umbilical vein endothelial cells. They found that silk fibroin-coated materials can support the normal growth and proliferation of vascular endothelial cells, and that the microstructure of the silk fibroin membrane surface can influence the morphology and biological behavior of these cells.

3.4.2. Bone Tissue

In recent years, there have been numerous reports on combining osteogenic seed cells with silk fibroin to create silk fibroin materials with bone repair capabilities. The silk fibroin in these materials provides the necessary space and environment for the growth, adhesion, and differentiation of bone cells. Yashi Jin [31] reported a method for preparing a 3D bone-like biomaterial with good bone repair effects, using ion diffusion to regulate the nucleation and growth of hydroxyapatite crystals in a 3D silk fibroin hydrogel. Bhardwaj et al. [32] seeded bovine chondrocytes onto a silk fibroin–chitosan three-dimensional scaffold and found that a silk/chitosan composite scaffold at a 1:1 ratio was most conducive to cartilage cell formation. Current silk fibroin biomaterials for bone repair mainly include silk fibroin films [33], silk fibroin nanofibers [34], silk fibroin hydrogels [31,35], and silk fibroin scaffolds [36-37]in several forms [38]. These silk fibroin biomaterials can be chemically modified and loaded with growth factors and inorganic compounds to promote osteoblast proliferation and differentiation in vitro and induce new bone formation in vivo. However, whether silk fibroin biomaterials can fully simulate the internal structure of bone tissue and be widely applied in clinical medicine still requires further in-depth research.

3.4.3. Skin Tissue

Due to its moisturizing and whitening properties, silk fibroin has long been widely used in cosmetics. Because of its water absorption, breathability, and other related biological activities, silk fibroin-based dressings exhibit the ability to accelerate skin regeneration, making it a popular material for research and product development in the field of medical aesthetics. Sheng et al. [39] prepared silk fibroin nanofiber mats loaded with vitamin E. These nanofiber mats have excellent waterproof properties, and the loaded vitamin E can be released stably. When mouse skin fibroblasts were cultured in vitro on these mats, cell growth and proliferation were significantly better than on glass slides. Guan et al. [27] prepared silk fibroin porous scaffolds (PSFSs)using freeze-drying. After implantation in vivo for 18 days, new tissue had already formed on the scaffold, with an almost identical structure to normal skin. Functional blood vessels were also found to be distributed proportionally. Inflammatory cells that infiltrated the PSFSs disappeared within 7

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days (compared to commonly used sponge scaffolds, where inflammatory cells and fibrous capsules were still visible after 18 days), indicating that silk fibroin porous scaffolds can promote the recovery of damaged skin and exhibit outstanding tissue compatibility.

3.4.4. Nerve

Silk fibroin-based nerve conduits can provide a favorable local microenvironment for nerve regeneration, acting as a bridging structure to guide and promote nerve regeneration, thereby repairing nerve defects [40]. Lorenz Uebersax et al. [28] used silk fibroin membranes loaded with nerve growth factor to control the gradual release of the growth factor during nerve cell growth, thereby continuously providing the necessary components for cell differentiation and promoting nerve regeneration. Yun Gu et al. [41] prepared an extracellular matrix-modified scaffold based on chitosan/silk fibroin to bridge a 10-mm gap in the rat sciatic nerve. Hematological and histopathological analyses demonstrated the safety of the scaffold, and the results showed good nerve regeneration in the rats after bridging.

4. Applications of silk fibroin in biomedical materials

4.1. Surgical sutures

For a long time, catgut sutures have been the primary absorbable surgical sutures used in medicine. However, catgut sutures have several drawbacks, such as poor flexibility, significant tissue reaction, and poor knotting performance when wet. Silk fibroin, on the other hand, has excellent knotting properties, good flexibility, and low tensile strength, making it less likely to cause tissue tearing. It is suitable for use in mucosal tissues or abrasion areas [42]. The Sericultural and Entomological Research Institute in Japan developed a soft silk surgical suture by coating silk with silicone. This suture does not cause any contamination to the body's tissues and is a safe medical suture [43]. In recent years, with the rise of the cosmetic surgery industry, the demand for cosmetic surgical sutures has increased significantly. These sutures need to be fine, uniform, and strong. The fibroin from fine-denier high-grade silk has become the main raw material for this purpose [44].

4.2. Immobilized enzyme carriers

Silk fibroin is a natural high-molecular-weight protein with a unique molecular structure, excellent mechanical properties, and good moisture absorption, thermal insulation, and antibacterial properties, making it an ideal material for preparing immobilized enzyme carriers[45].The application of silk fibroin as an immobilized enzyme carrier dates back to the 1970s.Graset et al. immobilized alkaline phosphatase on silk fibroin fibers through diazotization and adsorption, as well as cross-linking methods. Chen et al. [47] immobilized esterase on electrospun silk fibroin nanofiber membranes (SF NFMs) with an average fiber diameter of 180 nm and a thickness of 60 μ m. These SF NFMs were used to catalyze the production of geranyl butyrate through ester interchange reactions. The SF NFMs could load 10.7%of the enzyme, with a relative enzyme activity reaching 120%of that of free esterase. In addition to phosphatase and esterase, various enzymes such as glucose oxidase, superoxide dismutase, asparaginase, neutral protease, insulin, and peptides can also be immobilized on silk fibroin nanoparticles through bioconjugation methods [48].

5. Conclusion

For a long time, China's sericulture industry has followed the traditional path of planting mulberry trees, raising silkworms, producing cocoons, and reeling silk, resulting in a singular range of end products and a value chain that is highly susceptible to fluctuations in international raw silk prices. As the economy and society have developed, the disadvantages of the traditional value chain have become increasingly evident, and the scale of traditional sericulture has tended to shrink. The diversified use of sericulture that gradually emerged in the 21st century has achieved breakthrough progress in multiple fields such as food, pharmaceutical health care, and chemical industry, developing a series of products. This has significantly expanded the application fields of silkworm and mulberry resources and achieved remarkable economic benefits.

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The fibroin from domesticated silkworms is produced in large quantities and can provide abundant natural protein raw materials for medical health care and tissue engineering. With its excellent biological and mechanical properties, fibroin has broad application prospects in the biomedical field. It can be used alone or in combination with other materials (such as fibroin/polymer materials) to develop high value-added bio-based new materials and medical products. On the one hand, this can improve the medical health level and quality of life of residents; on the other hand, the newly expanded value chain is less affected by factors such as raw silk prices and can maintain a stable and sustainable demand for cocoons. While enabling enterprises to profit, it can also boost the enthusiasm of sericulturists, generating significant economic and social benefits.

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