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Review Article

Potential use of natural silk for bio-dental applications

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الملخص

هدف البحث: يعتبر الحرير بروتينا متعدد الجزيئات غزل إلى ألياف بواسطة ديدان القز والعناكب في البيئة المحيطة. ولقد استعمل الحرير كمادة حيوية بنجاح في عدد من التطبيقات الحيوية لعدة سنوات، في حين أن استعمالاته محدودة في طب الأسنان. إن الهدف من هذه المقالة هو استكشاف إمكانات وخواص الحرير الطبيعي للاستخدام في التطبيقات العلاجية للأسنان. تم تسليط الضوء على البنية والمزايا الأساسية التي تجعل من الحرير مقبولا حيويا ويمكن استعماله في الاجراءات العلاجية لطب الأسنان.

طرق البحث: أجري بحث في الأدبيات العلمية في قواعد البيانات لمواقع ميدلاين، وشبكة العلوم، وسكوبس، وايبيس لتوضيح الخواص الطبيعية للحرير وطرق تجهيزه للاستعمالات الحيوية الطبية والتطبيقات السنية العلاجية.

النتائج: للحرير خصائص طبيعية متميزة، مثل القوة ومقاومة الضوء والحرارة والرطوبة والمطابقة الحيوية. وبإذابة الحرير يمكن الحصول مواد مختلفة، مثل الأفلام والهلاميات والألياف والألياف المتناهية الصغر والحبيبات والرغوة والكرات والفرشات الكهربائية الصغيرة والمتناهية الصغر. والتطبيقات في مجال طب الأسنان تتضمن المعادن الحيوية والهندسة النسيجية للتطبيقات الهيكلية وتحرير الأدوية.

الاستنتاجات: إن هناك ازدياد ملحوظ في البحوث حول المواد المنتجة من الحرير، إضافة إلى التطبيقات العلاجية والحيوية المتوقعة مستقبلا بما فيها مجال طب الأسنان.

الكلمات المفتاحية: الهياكل; حرير العث الأسود; طب الأسنان; مواد طب الأسنان الحيوية

Abstract

Objectives: Silks are protein polymers that are spun into fibres by silkworms and spiders under ambient conditions. Silk has been used as a biomaterial in a variety of biological applications for many years, whereas there are few applications in dentistry. The aim of this study was to explore the potential properties of natural silk for dental applications by determining the structure and features that make natural silk a biocompatible candidate.

Methods: We conducted a literature search through the recognized databases of medline, ISI web of science, SCOPUS, and EBASE to elucidate the natural properties of silk, its processing for biomedical applications and its use in dental applications.

Results: Silk has excellent natural properties, such as strength, resistance to light, temperature and humidity and biocompatibility. Once silk has been dissolved, it can be used to produce a variety of materials, such as films, gels, fibres, nanofibres, granules, foams, spheres and electrospun mats, on a micro or nano scale. Applications in dentistry include biomineralization, tissue engineering for scaffold applications and drug delivery.

Conclusions: There has been renewed research on silk-based materials for various biomedical applications, including dentistry.

Keywords: Bio-dental materials; *Bombyx mori* silk; Dentistry; Scaffolds

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Introduction

Silks are protein polymers that are spun into fibres by silkworms and spiders under ambient conditions.[1] There are many silk-producing animal organisms, but most natural silk is obtained from silkworms.[2] Silk was discovered in China around 2700 BC and is cultivated in both Asia and Europe, but the main sources remain China, India and Japan.[3] Silk has a long history in biomaterial applications, as it has been used as a surgical suture material for decades.[4] More recently, it has been used in other biomedical applications, such as tissue engineering scaffolds[5] and drug delivery.[6,7]

Silk has the properties required for biomaterial applications, including biocompatibility, lack of toxicity and irritancy[8] and excellent mechanical properties.[9] It can perform under a wide range of conditions of humidity and temperature.[10] When used in tissue engineering scaffolds, regenerated *Bombyx mori* silk is compatible with human mesenchymal stem cells *in vitro*, and the scaffolds are comparable to collagen scaffolds. *In vivo*, the tissue inflammatory reaction to silk is similar to or less than that to collagen.[11] Silk proteins degrade slowly in biological environments,[12] which could be an advantage in dentin tissue regeneration. Additionally, silk can be manipulated to form a variety of materials and properties, e.g. films, fibres, hydrogels, foams and coatings on other materials, depending on the application.[13] Because of its unique properties, there is increasing interest in use of silk in biological applications.[14] There has been a dramatic rise in the number of scientific publications on this material, from 2206 in 1975–1979 to 21 166 in 2011–2013.

In dentistry, biomaterials are used to maintain and improve the quality of dental care. They can be used to prevent or treat disease, relieve pain or improve aesthetic appearance or functions such as speech and mastication. These objectives require materials to alter or replace lost or diseased tooth portions.[15] Many new materials have been developed for dentistry,[16] but ideal dental materials for all applications are still lacking. The aim of this study was to review the structure and properties of natural silk with respect to its potential use in dentistry and to help dental clinicians and postgraduate students understand the structure, properties and manipulation of silk for applications such as tissue engineering and dentin and periodontal regeneration.

Natural silk as a biomaterial

Natural silk fibres are composed of an inner protein core and an outer skin covered by a protective coating.[12] There are many silk-producing animal organisms, but most natural silk is obtained from two sources, spiders and silkworms (*B. mori*).[2]

Spiders can produce various types of silk for specific applications.[17] The mechanism of silk spinning by spiders is not fully understood; however, they can spin environmentally safe, stable, biodegradable silk fibres under ambient conditions using water-based protein solutions.[18] Spider silk is of interest to researchers because of its excellent mechanical properties; it is as strong as many synthetic fibres.[19] More than 34 000 different species of spider exist in nature; however, the cannibalistic nature of spiders makes cultivation of pure spider silk impossible.[13]

Structurally, *B. mori* silk consists of two protein components, sericin and fibroin.[13,20] Sericin is a glue-like water-soluble glycoprotein that binds the fibroin fibres, while fibroin is the structural protein of silk fibres and is insoluble in water.

Sericin, an amorphous glycoprotein, accounts for 20–30 wt% of *B. mori* silk.[21] It has a relative molecular mass of 10–300 kDa, is rich in serine and is secreted by paired glands in silkworms.[22] Sericin plays a functional role in coating and adhering the twin filaments and cocoons[23] to protect silk from microbial degradation, animal digestion and environmental damage.[12] It has other surprising properties, such as resistance to ultraviolet light and oxidation and an ability to absorb and release moisture easily.[22] Sericin is composed of 18 amino acids,[22,24,25] 80% of which are composed of serine, aspartic acid and glycine, giving it good moisture-absorbing properties.[25] The relative composition of amino acids may vary (Table 1), depending on the source of silk and processing method.[22,24–26] Sericin is soluble in water because of its high content of hydrophilic amino acids (about 70%). Large sericin peptides are soluble in hot water, while small peptides can be dissolved in cold water.[22] Because of the proven role of sericin in inducing allergic and immunological reactions,[27] all sericin must be removed before use for any biological application.

Silk fibroin is the structural protein of silkworm silk fibres and is insoluble in many solvents, including water.[13] Silk fibroin is a large protein macromolecule made up of more than 5000 amino acids;[29,30] it accounts for about 75 wt% of total *B. mori* silk.[21] Silk fibroin comprises both a crystalline region (∼66%) and an amorphous (∼33%) region.[31] The crystalline portion is composed of repeating units of glycine, alanine and serine, typically $[G-A-G-A-G-S]_n$, and forms a β-sheet structure in the spun fibres, which is responsible for its good mechanical properties.[31] The amorphous region consists mainly of the amino acids

Table 1: Proportions (%) of amino acids in sericin.

Amino acid	Zhang[22]	Wu[24]	Sothornvit[25]	Vaithanomsat[26]
Serine	28	27.3	32.74	31.99
Aspartic acid	17.97	18.80	17.64	15.74
Glycine	16.29	10.70	9.89	14.20
Glutamic acid	6.25	7.20	7.31	6.28
Arginine	3.52	4.90	6.16	4.29
Threonine	7.78	7.50	5.51	7.73
Tyrosine	2.87	4.60	4.63	3.01
Alanine	5.20	4.30	3.86	4.85
Valine	3.77	3.80	3.14	3.30
Lysine	1.21	2.10	3.05	4.17
Histidine	1.32	1.70	1.81	1.49
Leucine	1.21	1.70	1.44	0.96
Isoleucine	0.64	1.30	1.04	0.72
Phenylalanine	0.64	1.60	1.08	0.37
Proline	–	1.20	0.59	0.71
Methionine	0.79	0.50	0.11	–
Cystine	0.69	0.30	–	0.20

Table 2: Amino acid composition of silkworm (*Bombyx mori*) silk fibroin.

Amino acid	Residue (%)
Glycine	44.7
Alanine	25.7
Serine	11.9
Tyrosine	5.4
Valine	2.4
Aspartic acid	1.6
Phenylalanine	1.6
Glutamic acid	1.1
Threonine	1.0
Isoleucine	0.6
Leucine	0.5
Proline	0.5
Arginine	0.5
Lysine	0.4
Histidine	0.2

From reference 28.

Table 3: Differences between spider silk and silkworm silk.

Feature	Spider silk	Silkworm silk
Silk types	Up to 6[13]	One[13]
Glands	Multiple glands near anus[38]	Secreted via mouth[39]
Domestication	Not possible[13]	Domesticated[13]
External coating	Glycoprotein[9]	Sericin[9]
Protein	Spidroin[12]	Fibroin[12]
Size	275–320 kDa[13]	350 kDa[13]
Sequence (motif)	GPGGX, GPGQQ $(A)_n$[40]	GAGAS, GAGAY[41]
Glycine (G)	37%[40]	46%[41]
Alanine (A)	21%[40]	29%[41]
Serine (S)	4.5%[40]	12%[41]
β-Sheet	30% (major ampullate)[9]	40–50%[42]
Hydrophobic contents	Variable, rich in glycine[43]	Hydrophobic residue[41]
Contraction in water (%)	44 ± 2 (major ampullate)[44]	0 ± 1 (water resistant)[44]

phenylalanine and tyrosine, with large side chains that are responsible for the hygroscopic properties of the material.[32] The proportions of amino acids in silk fibroin have been known for more than 60 years (Table 2). For a long time, silk fibroin was considered a single large polypeptide; in 1976, however, Shimura et al.[33] demonstrated that it is composed of at least two protein subunits, a heavy chain (H fibroin, relative molecular mass ~350 kDa) and a light chain (L fibroin, ~25 kDa), which are attached to each other by disulfide bridges. Another component of silk fibroin is glycoprotein P25 (~30 kDa), which is attached by non-covalent interactions to the covalently bonded heavy and light chain complex.[34,35] Quantitatively, H fibroin, L fibroin and P25 are present in silk fibroin in a molar ratio of 6:6:1,[34] suggesting that P25 is attached to a set of six H–L fibroin dimers.[34] Glycoprotein P25 is secreted with H fibroin[35] and is considered important in maintaining the integrity of silk fibres; however, its role in the formation of silk fibroin is not yet clear.[34]

Both silkworm and spider silks are composed of glycine-rich proteins, and they share many properties;[12] however, silk produced by silkworms differs in composition, structure and properties (Table 3).[36,37]

Processing of natural silk for biomedical applications

For textile applications, little processing (e.g. dying or spinning) of natural silk is needed;[45] however, biomedical applications require a specific morphology at a micro or nano scale. Natural silk fibres cannot be transformed directly and must first be dissolved, although they are difficult to dissolve, even in harsh solvents, because of their complex structure and the presence of strong hydrogen bonding.[46] Once silk has been dissolved, it can be used to produce a variety of materials such as films, gels, fibres, nanofibres, granules, foams, spheres and electrospun mats.[13,46] The three main stages in the processing of silkworm silk for biomedical applications are: removal of sericin (de-gumming), dissolution in aqueous ionic solutions and fabrication of biomaterials with regenerated silk.

De-gumming of silk cocoons

The first stage is known as "de-gumming",[47] "pre-treatment" or "desericinization".[33] Sericin must be removed because of it induces allergic and immunological reactions.[27] The aim is to remove sericin from silk cocoons without degrading silk fibroin.[13,46,47] A variety of methods has been used, such as boiling silk cocoons in hot water or weak sodium bicarbonate solution for 30–60 min.[20,33] There is some controversy concerning this technique, as some researchers consider that they have no effect on silk fibroin,[48] while others have found that heating at high temperatures results in molecular degradation.[33,47]

Dissolving silk fibroin in aqueous ionic solutions

The presence of strong intermolecular hydrogen bonding and highly organized clusters of β-sheets in silk fibroin makes dissolution difficult.[46,49] Methods involving high concentrations of ionic solutions[13,50,51] have been used.[52] Both cations and anions can affect the solubility, but a higher ratio of anions has been found to increase the solubility of silk fibroin.[13] Commonly used aqueous ionic solutions for dissolving silk are concentrated lithium bromide, lithium thiocyanide and calcium chloride. These solutions can be dialysed against deionized distilled water to remove inorganic ions and obtain aqueous silk solutions.[52]

Fabrication of silk biomaterials

A variety of biomaterials can be fabricated from either aqueous silk solution or lyophilized regenerated silk by techniques such as sol–gel, ion leaching and electrospinning. Polymer nanofibres remain an important type of nano-biomaterial, with a wide range of applications in biotechnology. Electrospinning is a popular method for synthesizing nano-scale fibres for tissue engineering and dentin regeneration applications,[53] because it can form continuous fibres, is easy to use and is cost-effective.[32] It can be used for making both polymers and composite fibres by blending with additives such as particles, antimicrobials or enzymes to obtain the desired properties.[53] These properties make electrospun fibres excellent candidates for a variety of biomedical applications, such as topical or parenteral drug delivery, wound dressing and scaffold materials for tissue engineering.[54] Nanofibres can facilitate packing of maximum volume fractions by controlling the fibre alignment and orientation, hence improving the material strength.[55] Desired scaffold properties, such as surface morphology, porosity and geometry, can be tailored by varying the electrospinning parameters[56] and can be functionalized for applications such as bioactive agents for biomedical applications.

Silk is highly biocompatible for a range of applications and well suited for cell culture purposes as well as adhering to fibroblasts for their proliferation.[57] Silk proteins are resistant to changes in temperature and humidity and can perform under a wide range of conditions. They degrade slowly in biological environments, which can be an advantage or disadvantage, depending on the application.[12]

Why are new materials required for dental applications?

The science of dental materials has developed remarkably in recent years, resulting in a wide range of new materials on the market. Continuous research is still needed, however, for the following reasons.

None of the available materials is ideal

Despite better understanding of materials chemistry and improvements in physical properties, no material has been found that is ideal for all dental applications.[15] For example, there has been concern for many years about mercury in amalgam restorations.[58] Another issue is the colour of amalgam for aesthetic reasons, and alternative materials are required.[59] Composite restorative materials are promising aesthetically, but they are less promising as amalgams mechanically and are sensitive to the technique of the dentist.[60] Nature has arranged complex biominerals in the best way, from the micro to the nano scale, and no one has yet managed to combine biological and physical properties to obtain ideal structures. In addition, no synthetic material can be intelligent enough to respond to external stimuli and react like natural tissues.[61] For example, the best option for replacing lost dentin tissue is regenerated dentin, which closely resembles natural dentin and responds to stimuli accordingly.

A large global market

There is a huge global market for modern dental materials. The dental composite resins developed recently gained a market of €550 million a year in 2005.[60] According to estimates,[1] there is a rapid increase in the use of dental implants, with more than 300 000 implanted every year, some patients having more than 12. The market for dental implants in the United States alone was US$ 910 000 in 2000.[1]

Many other factors can increase the demand for biomaterials generally, such as greater awareness of patients, better life style, increasing populations and longer life expectancy. For instance, the average life span in the USA increased by 30 years during the 20th century[62] and is expected to increase to 100 years by 2050.[63] The number of centenarians is increasing every year, with an 8.5% rise per year in Australia.[64] Although there were only 2835 centenarians in Mexico in 1930, the number had gone up to 19 000 in 2000 and was expected to increase to more than 137 000 in 2050.[63] It can therefore be assumed that there will be an increasing demand for all biomaterials, including dental materials, to maintain the quality of life of older populations.

Maintaining tooth vitality

As dental pulp plays an important role for teeth, dental professionals try to keep the pulp vital for as long as possible. Loss of pulp tissue (tooth vitality) changes the physical properties of dentin, and the tooth may behave differently from a vital tooth.[65] The water content of dentin may decrease by up to 9%,[66] and tooth colour may change due to the presence of blood breakdown products, which may not be acceptable aesthetically.[65] If teeth are treated by endodontics, the chemicals used in the preparation make them weak mechanically and prone to fracture.[67] Hence, maintaining pulp vitality enhances the physiological life of a tooth *in situ*. This can be achieved by biomimetic approaches and tissue regeneration, in which lost dental tissues are replaced by natural dental tissues.

Potential of silk biomaterials for dental applications

Silk biomaterials have been used in biomedical applications for many years; however, there are only a few applications in dentistry, such as for suture materials and dental tissue regeneration. Silk has been used for tissue regeneration alone or in combination with other materials as composite materials, and novel nano-composites have been developed from silk proteins and silica, combining the beneficial properties of the two components.[68] Similarly, silk–hydroxyapatite composites have been synthesized by blending hydroxyapatite nanoparticles into silk solutions.[69]

Minerals such as hydroxyapatite are an essential component of tooth enamel and dentin[70] and account for the toughness and hardness of these tissues. The process of hydroxyapatite nucleation and its crystal structure are controlled by organic macromolecules such as osteonectin, phosphoproteins and dentin matrix protein 1 (DMP-1). DMP-1 plays a major role in controlling the nucleation, growth and morphology of hydroxyapatite crystals in dentin.[71] It is also found in the cells of other hard tissues, such as ameloblasts, osteoblasts, osteocytes and cementoblasts. A biomimetic approach to producing biominerals has been used, involving a combination of spider silk and DMP-1. For this purpose, a novel spider-like domain and a domain of DMP-1 were cloned and expressed, and the two domains were then used for self-assembly and nucleation of hydroxyapatite. Because of the unique features of the silk domain and the hydroxyapatite nucleation ability of the DMP-1 domain, this combination has great potential for tissue engineering of biomineralized tissues.[72]

Tissue engineering and regenerative medicine are active areas of research for regenerating human tissues, including oral and dental tissues. Silk-based composite scaffold materials are being used for biomineralization studies of hydroxyapatite, and a template from natural spider silk was used for nucleation and growth of hydroxyapatite crystals.[73] Similarly, silkworm silk films with a β-sheet conformation (induced with methanol) resulted in the growth of hydroxyapatite within a few hours of incubation in simulated body fluid.[74] These findings suggest that silk-based materials are compatible and have the potential for nucleation and crystal growth of hydroxyapatite, an essential element of biomineralization. Similar applications of silk materials can be expected in the future. In addition, silk has been used in tissue engineering of scaffold applications because of features such as a highly variable morphology, good physical and mechanical properties and biocompatibility.[13]

Recently, there has been an increase in the use of coating technology for biomaterials to improve their surface properties. From the biological point of view, material coating could enhance or reduce cellular adhesion for biomedical implants.[13] The biocompatibility and low immunogenicity of natural silk make it a good candidate for coating applications. In addition, antibacterial properties can be included in silk-based composite materials, such as silk nano-composites containing silver nanoparticles.[75] Titanium nanoparticles have been shown to stop the growth of microorganisms such as *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.[76]

Damrongrungruang et al.[77] characterized electrospun silkworm silk scaffolds for gingival tissue regeneration applications and found that gingival fibroblasts attached and proliferated on electrospun fibres, confirming the non-toxicity of silk. In a study with mouse fibroblast cells *in vitro*, polypropylene and polyamide foams coated with silkworm silk supported the cells and allowed them to proliferate.[78]

Conclusions

Silk is a natural material with many favourable properties, such as biocompatibility, adaptable mechanical properties and stability under a wide range of conditions of humidity and temperature. Silk materials can be tailored to various forms of architecture and morphology, depending on the application, suggesting their potential for dental applications, such as in dentin and periodontal tissue engineering.

The literature reviewed suggests that silk has wide scope in dental applications. Extensive collaborative research involving multiple disciplines is required. It is hoped that continuous research funding and hard work by biomaterial scientists will open up a new range of opportunities for silk-based materials for biomedical and dental applications, including periodontal tissue regeneration, engineered scaffolds for alveolar bone, synthetic bone grafts with bioactive glass, hydrogel delivery of drugs and macromolecule signals to dental pulp.

Conflict of interest

None.

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