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SILK FIBROIN AND POTENTIAL USES IN REGENERATIVE DENTISTRY - A SYSTEMATIC REVIEW

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Abstract

Silk fibroin is an organic polymer isolated from cocoon silk fibers. Recently it has been studied as a substrate for tissue engineered cartilage, bone , ligaments, nerves, cornea and also for drug delivery applications. The current review focuses on recent advance in silk fibroin and its potential uses in regeneration therapies, mainly in the dental field. Data extraction was carried out according to the standard Cochrane systematic review methodology and the following databases were used: PubMed, Google Scholar, Medline and the Google library. Out of the 151 related articles that were critically assessed, only 57 articles were included in the critical appraisal. There is evidence that silk fibroin is a biocompatible polymer and has been proved to be cytocompatible with a wide variety of cells. Composite silk fibroin with hydoxyapaptite, bioglass, gold or silica can be used in a variety of applications. Regenerative dentistry may profit from the silk fibroin due to possible future uses in implant therapy, mineralized tissue formation or healing of the wounds of the buccal mucosa.

Keywords: silk fibroin, organic polymer, bone regeneration, drug delivery, wound healing

1.Silk fibroin

Silk represents the strongest and toughest naturally occuring polymer material (1) . Silk from silkworms and orb-weaving spiders have impressive mechanical properties in addition to environmental stability, biocompatibility, controlled proteolytic biodegradability, morphological flexibility and the ability for the aminoacid side charge modification to immobilize growth factors (2). Silk fibroin is a structural protein isolated from cocoons silk fibers of the silkworm Bobymex mori (2, 3) and it has a long history of use in clinical applications as sutures (2). Recently it has also been studied as a substrate for tissue engineered cartilage, bone (4), (5), (6), ligaments and also for drug delivery applications (7), (8-10).

1.1.Silk fibroin in drug delivery vehicles

A wide range of polymeric materials have been investigated for use as drug delivery matrices, including biodegradable synthetic polymers such as PLGA, and natural polymers like collagen (3). The addition of silk seemed to improve the controlled release proprieties. It was shown, that the more the crystalline content of silk increased ,the slower was the release of the encapsulated protein (7). Other strategies to fine-tune the release from silk fibroin matrices include the embedment of drug loaded micro- or nanoparticles or the coating of micro- or nanoparticles with silk fibroin films (11). Also silk coating of liposomes loaded with the anti-tumor drug Emodin significantly retarded drug release without affecting the drug efficacy (9). Moreover silk microspheres could offer unique options as drug delivery carriers,given the fact that silk microspheres are much smaller than PLGA microspheres (3).

1.2 Silk fibroin scaffolds

Mimicking the natural extracellular matrix is one of the critical and challenging technological barriers, for which scaffold engineering has become a prime focus of research

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within the field of tissue engineering (12). Silk fibroin could act as a scaffold material, a substrate on which cells can thrive and receive stimuli such as growth factors that guide the differentiation process of cells (7), through controlled drug delivery. The compressive modulus of gas foamed silk fibroin scaffolds was superior as compared to scaffolds prepared from collagen, chitosan, PLGA and PLLA, materials that are intensively studied as scaffolds materials (13).

A variety of cells were cultured on fibroin scaffolds and maintained their viability. Endothelial cells were cultured on the silk fibroin and the cells attached and proliferated, forming after a week microvessel-like structures (14). Also a silk fibroin film and BMP-2 induced osteogenic differentiation of human bone marrow stromal cells (5). Additionally, amniotic fluid stem cells were also seeded on silk bioengineered constructs and were able to differentiate into osteoblast cells in vivo (15). In vitro culture in chondro- or osteo-inductive media showed that silk non-mulberry constructs pre-seeded with human bone marrow stromal cells exhibited prominent areas of the neo tissue containing chondrocyte-like cells, whereas mulberry constructs pre-seeded with human bone marrow stromal cells formed bone-like nodules (16).

Silk fibroin nanofibers seem to be cytocompatible with human keratinocytes and fibroblasts (4, 6). Three forms of silk fibroin matrices, woven (microfiber), non-woven (nanofiber), and film form, were used to test the compatibility with cell cultures of normal human oral keratinocytes by examining the cell attachment and spreading of cells (17-19). The results indicated that the silk fibroin nanofiber matrix may be preferable to silk fibroin film and silk fibroin microfiber matrices for biomedical applications, such as wound dressings and scaffolds for tissue engineering (17), (18).

Moreover composite silk scaffolds, such as nanostructured biocomponent silk fibroin/chitin scaffolds proved to be cytocompatible in interaction with human epidermal keratinocytes (20). Osteoblasts were cultivated on silk fibroin/nano-hydroxyapatite scaffolds in vitro and demonstrated excellent cytocompatibility as well as improved viability of osteoblasts (21).

Besides hydroxyapatite, collagen was incorporated into silk fibroin and therefore biomimetic bone substitutes of of collagen-silk fibroin/hydroxyapatite were fabricated (22). This bi-template material exhibited good biocompatibility and stimulated the bone marrow mesenchymal stem cells to differentiate into the osteoblast cell lineage (22).

1.3.Biocompatibility of silk fibroin

Being a protein,biodegradation of silk fibroin predominantly occurs through proteolytic enzymes,with non-toxic degradation products and unproblematic degradation in vivo. Silk scaffolds have low immunogenicity, when the immunogenic and glycosylated proteins are separated (23). The use of silk fibroin films in wound repair led to significantly lower distribution of inflammatory neutrophils than in controls, with animal models (24), while the implantation of a silk fibroin scaffold subcutaneously in mice led to a mild inflammatory reaction that disappeared after 12 weeks (25). No evidence of any inflammatory reaction was seen when a nanofiber silk fibroin membrane was used for 12 weeks in rabbit calvarial defects (26) .

2.1 Applications of silk fibroin

Due to its ease of processing, excellent biocompatibility, remarkable mechanical properties and tailorable degradability, silk fibroin has been explored for fabrication of various articles such as films, porous matrices, hydrogels, nonwoven mats and has been investigated for use in various tissue engineering applications, including bone, tendon, ligament, cartilage, skin, liver, trachea, nerve (27), cornea (28), eardrum, dental, and bladder (12).

Because silk fibroin can be gelatinized and still retain its biocompatibility and permeability,it can be used in a variety of applications (29). Silk fibroin scaffolds were used in wound-healing processes (2), (30). Silk fibroin gel containing electrically polarized hydroxyapatite is an effective wound dressing and effectively advanced the maturation of fibroblasts porcine cells (29). Moreover the addition of hydroxyapatite or polarized hydroxyapatite to the silk fibroin scaffolds improved the wound-healing properties, enhancing the migration of the endothelial cells, and so the number of migrated cells was 1.5 times higher than on the silk fibroin scaffold alone (30).

So far, the main focus of silk fibroin drug delivery systems has been on tissue regeneration applications (11). Other strategies to fine-tune the release from silk fibroin matrices comprise the embedment of drug loaded micro- or nanoparticles or the coating of micro- or nanoparticles with silk fibroin films (11). For instance, growth factor loaded silk fibroin scaffolds were suggested for the tissue engineering of bone and cartilage, as well as for vascular and nerve regeneration devices and wound healing products. Moreover, silk fibroin matrices were proposed for oral, transmucosal and ocular drug delivery (11).

Silk fibroin could be also used in nerve regeneration techniques and could be helpful in restoring motor function and preventing abnormal sensations after nerve injury (27). A nerve guidance conduit using electrospun silk fibroin was implanted in a 10-mm defect of the sciatic nerve in rats and the immunostaining analysis showed the formation inside the electrospun silk fibroin of well myelinated nerve fibres stained with axonal neurofilament and myelin basic protein (27).

Scaffolds of silk fibroin were used for ligament tissue engineering applications (31), (32). Mesenchymal stem cells were seeded on a hybrid scaffold, comprised of knitted silk fibroin and aligned silk fibroin electrospun fibers, and led to the expression and production of ligament-related proteins (32).

2.2 Silk fibroin in bone regeneration

Silk fibroin scaffold may be a good substitute for bone regeneration with better results than a commercially available polylactic acid scaffold (33). In vitro study showed that silk fibroin led to increase activity of the obsteoblasts than polylactic acid, while the in vivo tests revealed that the silk fibroin scaffold regenerated 78.30% of the original bone volume, while the polylactic acid implantation led to only 49.31% bone formation in rats (33). Also silk fibroin hydrogels injected in critical-sized defects in rabbits resulted in greater trabecular bone volume and thickness,significantly higher mineral and rate of bone formation when compared to PLGA - copolymer of polylactic-polyglycolid acid (34).

Not only scaffolds, but also silk fibroin membranes proved bone regeneration abilities in animal studies (35). Silk fibroin nanofiber membranes were implanted in calvarial defects of rabbits for guided bone regeneration and resulted in complete healing with new bone at 12 weeks (26). After one month implantation of silk-fibroin subcutaneously in mice the three-dimensional soft tissue augmentation was stable, and histologic analysis revealed revascularization of the area through the biomaterial (25). In another animal study on rabbits, low-molecular-weight silk fibroin with Choukroun platelet-rich fibrin were used and led to 59.83 ± 10.92% new bone formation, compared to 49.86 ± 7.49% in the control group, while the tissue mineral density was slightly increased (36).

Also small fragments of silk fibroin are able to increase the expression of osteoblastogenic genes and DNA microarray results showed that alkaline phosphatase collagen type-I alpha-1, fibronectin, and transforming growth factor-beta1 expressions significantly increased (37).

Silk fibroin sponges were used to support orthopedic regeneration using fibrin gels loaded with growth factors and human adipose -derived mesenchymal stem cells (38). This construct had angioinductive, as well as osteoinductive abilities, proven by the the deposition of bone matrix proteins, alkaline phosphatase activity and calcium deposition, along with the formation of vascular networks, evidenced by endothelial cell surface markers (38).

Aquous-based silk fibroin scaffolds with plasma irradiation were succesfully tested in 48 femur critical size defects and led to the formation of new bone around the scaffolds (39). The introduction of plasma helped to change the hydrophobic nature into hydrophilic (39). Moreover the immunohistochemical examination revealed the increased expression pattern in a set of osteoblast specific genes (TGF-β, TGF-β type III receptor, Runx2, type I collagen and osteocalcin) (39).

Silk fibroin scaffolds of different sizes and osteogenic cells seeded were used in different experiment to study the differentiation of the human mesenchymal stem cells along the osteoblastic lineage (40), (41). Silk fibroin scaffolds seeded with human stem cells showed good results in bone regeneration in animal studies (42). A study on cranial bone defects in rats used silk fibroin scaffolds seeded with human stem cells from dental pulp and amniotic fluid stem cells. After 4 weeks of implantation mature bone correction with higher bone amount produced by the human amniotic stem cells was observed (42). Also silk scaffolds seeded with human mesenchymal stem cells predifferentiated in a osteogenic medium, seemed to promote bone formation in cranial defects in mice (43). Furthermore, silk scaffolds with human mesenchymal stem cells that had previously been differentiated along an osteoblastic lineage in vitro, were implanted in femur defects in rats, and resulted in an osteoinductive effect. The defects were completely bridged with a callus on the outside and around 30% newly formed woven bone tissue inside the defect (1). According to a study that investigated the human bone marrow derived mesenchymal stem cells, the mineralization on silk fibroin scaffolds with pores of 112-224 μm diameter was most efficient with an initial cell pre-culture period of 9 days (41).

By biomimetic strategy, apatite-coated porous biomaterial based on silk fibroin scaffolds might provide an enhanced osteogenic environment for bone-related outcomes (44). Autologous bone marrow stromal cells seeded on apatite-silk fibroin scaffold managed to completely repair the bony defects in a mandibular canine model (44). However, when silk fibroin scaffold or apatite-silk fibroin were used alone the bony defects remained in the centre with undegraded silk fibroin and fibrous connective tissue (44), showing the boosting of the regeneratory effect when bone marrow stromal cells were added.

However, silk is not an osteogenic material and has a compressive stiffness significantly lower than that of native bone (45) . Hydroxyapatite (HA)-silk fibroin scaffold were designed to induce and support the formation of mineralized bone matrix by human mesenchymal stem cells enhancing the formation of tissue engineered bone by two mechanisms: through osteoconductivity of the material leading to increased bone matrix production, and by providing nucleation sites for new mineral resulting in the connectivity of trabecular-like architecture (45).

Table1. Applications of silk fibroin scaffolds in osteo-dental regeneration.

Tissue Regenerated	Scaffold	Cells	Year /Study
cartilage-like tissue	silk fibroin scaffold	human mesenchymal stem cells	(4)
bone-like tissue	silk fibroin scaffold	human bone marrow stem cells	(6)
ligament	silk fibroin scaffold	human periodontal ligament fibroblasts	(31)
neo-osteochondral tissue	silk fibroin scaffold	human bone marrow stromal cells	(16)
mineralized dental tissue (osteodentine)	silk fibroin scaffold	rat tooth bud cells	(52)

However, in another study the nano-hydroxyapatite alone resulted in significantly higher bone regeneration than the grafting with the combination of silk-fibroin and nano-hydroxyapatite (46). In New Zealand calvarial defects of white rabbits treated with nano-hydroxyapatite showed 40.16% ± 8.27% new bone formation compared to 16.62% ± 3.05% in the hydroxyapatite graft with silk fibroin scaffold (46). Additionally in the same study, even the control led to better results, (25.66% ± 10.98%) than the silk fibroin group (46). Other authors supported the ideea that the hydroxyapatite/ silk fibroin scaffold could be used with better results than the hydroxyapatite scaffold alone (47). The composite hydroxyapatite/regenerated silk fibroin scaffold supported a significantly increased alkaline phosphatase activity and cell viability than the hydtoxyapatite scaffold alone (47). Also composite silk scaffolds with nano-hydroxyapatite crystals indicated good results in bone regeneration (21).

Composite bioglass/silk fibroin scaffolds may have future uses in the treatment of the osteoporotic fractures, being able to support regeneration through sustained release of PDGF-b and BMP-7 incoporated in this composite scaffold (48). The ability for these scaffolds to be degraded over time and initiate bone turnover/remodeling has been shown (48).

Moreover, nanocomposites of silk nanofibers and gold nanoparticles were fabricated and the resulting scaffolds led to increased cell size of the human mesenchymal stem cells cultivated on them (49). Composite silk-silica biomaterials for bone regeneration were also fabricated and the addition of silica upregulated the osteogenic markers bone sialoprotein and collagen type 1 in human mesenchymal stem cells subjected to osteogenic differentiation (50). Human mesenchymal stem cells also adhered, proliferated and differentiated towards osteogenic lineages on composite silk/ silica films (50).

2.3 Silk fibroin in dental applications

Silk-gel material is a promising biomaterial for periodontal and maxillofacial therapies, either as a scaffold for cells or alone as a biomaterial (25). In regenerative dentistry, stem cell-based therapy often requires a scaffold to deliver cells and/or growth factors to the injured site (51). Silk fibroin is a promising biomaterials for tissue engineering as it is non toxic and promotes cell proliferation (51).

Based on the successful use of silk scaffolds in bone tissue engineering, researchers examined their utility for mineralized dental tissue engineering and found that tooth bud rat cells seeded onto silk scaffolds appered to guide mineralized tissue formation of osteodentin (52).

Human dental stem cells obtained from peridontal ligament were cultured on fibroin films and showed discrete proliferation as well as the mantainance of the level of expression of the mesenchymal stem markers CD73, CD90 or CD105 (51). Moreover the combination of human dental stem cells on fibroin and graphene oxide -bioengineered construct has a strong potential for a future therapeutic use in regenerative dentistry (51).

A novel hierarchical textile structure made of silk fibroin from Bombyx mori was developed for ligament regeneration (31). For this purpose, human periodontal ligament fibroblast were cultured in direct contact with the silk structure and therefor, demonstrated an increased secretion of aggrecan and fibronectin at 3 and 7 days of culture, and no change in IL-6 and TNF-α secretion (31). Although the study tried to regenerate the anterior cruciate ligament, the positive results of dental ligament fibroblast lead way to future dental ligament regeneration.

Silk fibroin can be used in implant therapy as a recent (2014) study suggests (53). Silk fibroin from non-mulberry source was immobilized on titanium surface, which led to improved cell adhesion and differentiation, facilitating a better osteogenesis on orthopedic implants (53). Peri-implant defects can be successfully repaired using silk fibroin powder mixed with Choukroun platelet-rich fibrin (PRF) (54). The study on rabbits concluded that after inserting dental implants and filling the peri-implant defects with silk fibroin powder and Choukroun PRF, the mean new bone formation is statistically significantly higher than the one of the control (54). Another silk fibroin and 4-hexylresorcinol incorporation membrane was fabricated to fill peri-implant defects and was succesfully used in rabbits (55). The silk fibroin and 4-hexylresorcinol membrane lead to 18.3 ± 1.9 mm mean bone regeneration, almost double than the control group that showed 9.3 ± 0.9 mm of new bone in the histomorphometric analysis (55). Moreover, premineralized silk scaffold was used as a substrate for bone marrow stromal cells to construct tissue-engineered bone for mandibular bony defects in a vrat model (56).

Tissue engineered buccal mucosa was obtained from oral keratinocytes and autologous canine fibroblasts seeded onto silk fibroin matrices (57) and the oral keratinocytes and fibroblasts exhibited good biocompatibility with the silk fibroin matrices (57). Additionally there were develloped silk fibroin materials for wound repair in the buccal mucosa (24). Ninety wounds to the bucal mucosa applied to ratss were treated with silk fibroin films and scaffolds. the wound shrinkage was significantly lower as well as the growth of mucosal epithelial cells was enhanced without any local or systemic immunological incompatibility (24).

In summars , silk fibroin is a promising material for future regeneration techniques in medicine. Dentistry may very well profit a lot from the different silk fibroin derived materials which are in tests nowadays since a vast majority of the studies concentrate on the regeneration of the osteo-dental tissues.

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