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Silk – A versatile Biomaterial for various Applications in Tissue Engineering and Regenerative Medicine

107 - Translationale Gesundheitsforschung – Brücken bauen von der Grundlagenforschung zu angewandter Forschung

Abstract

In this scientific talk, the use of silk, i.e. the core protein of *Bombyx mori* silkworm thread, as biomaterial for various applications in tissue engineering and regenerative medicine is discussed based on our own studies on the topic. Despite its frequent use as a suture material, tissue engineering and regenerative medicine have neglected silk as a biomaterial due to concerns about its biocompatibility and degradation. Beside its safety numerous studies have demonstrated its outstanding characteristics as a material for biomedical applications in the last few years. Especially its mechanical toughness/resilience based on the molecular structure, its slow rate of degradation and high processability have led to the use of silk in regenerative approaches of different tissues. In our own studies we have focused on the generation of anterior cruciate ligament grafts, peripheral nerve guidance conduits and abdominal wall hernia mesh via textile-engineering technologies. Moreover, we also worked on the defined modifications of silk, e.g. to tailor its biological interaction with cells and on the use of silk to create artificial tissue-mimicking 3D culture systems. Importantly for our students, the established know-how on this topic is constantly transferred from current research projects into various courses.

Silk fibroin has come a long way from ancient China and the Silk Road to today’s regard as a promising candidate for a wide range of applications in the field of tissue engineering and regenerative medicine.

Keywords:

Tissue Engineering, Silk proteins, Biomaterial, Scaffold, Implant

Silk fibroin is produced by all spiders and many insects. Worldwide, the most commonly used silk fibroin is derived from the cultivated *Bombyx mori* silkworm. For over 5,000 years, the main focus has been on clothing purposes but it has also been used for various other applications including cosmetics or production of pharmaceuticals. The development of new technologies has substantially increased the quality of silk products and facilitated their use in biomedical applications, such as sutures.

Historically, silk was used for thousands of years as suture material along with other naturally-derived materials such as catgut, cotton, or linen. In modern medicine, silk sutures have been used since the end of the nineteenth century (Moy, Lee, & Zalka, 1991), but the record of several cases of inflammatory response and immunogenic reactions (Kurosaki et al., 1999; Nebel, Rosenberg, Tobias, & Nathan, 1977; Peleg, Rao, & Emrich, 1986; Soong & Kenyon, 1984) to silk sutures has led to its replacement in many medical areas by sutures of synthetic polymers. A review by Altman (Altman et al., 2003) revealed that the adverse effects of raw silk (also known as black braided silk) can be attributed to sericin. In several studies sericin-deprived silk elicited only very mild inflammatory responses *in vivo* (Fan, Liu, Wong, Toh, & Goh, 2008; Wang et al., 2008; Yan, Zhao, Wang, Gu, & Yang, 2009) even lower than responses observed with traditional biomedical materials such as PLA (polylactic acid) or collagen (Acharya, Hinz, & Kundu, 2008; Meinel et al., 2005).

Despite its frequent use as a suture material, tissue engineering and regenerative medicine have neglected silk as a biomaterial due to concerns about its biocompatibility and degradation properties. In the last few years numerous studies have demonstrated the safety of this material for biomedical applications. Silk is a FDA-approved material for medical products. After removal of sericin from the surface of the raw silk fibroin fibers in the so-called “degumming” process, they can be used in their native fibrous structure or can be dissolved and post-processed in multiple ways.

At the UAS Technikum Wien, various processes have been established to generate a host of biocompatible scaffold structures like hydrogels, sponges, films, particles or textile engineered (braiding, knitting, weaving) scaffolds.

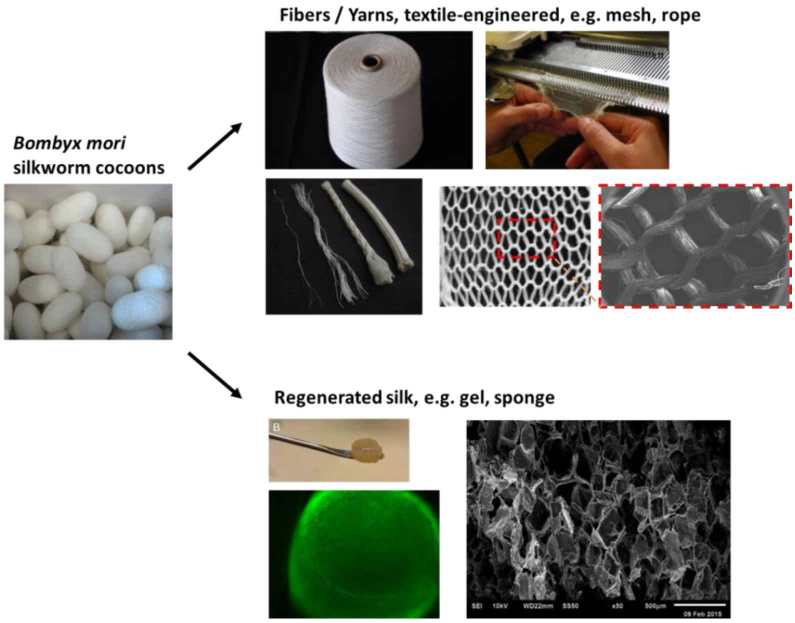

Figure 1: *Bombyx mori* silkworm cocoons as a biomaterial source. Silkworm cocoons can be processed into biomaterials via two main routes. First the raw fibers of the silkworm cocoons can be textile-engineered into different geometries. In the figure two examples of a wire-rope design (from single fibers to strands enveloped by a sheathing structure with different braiding design) and meshes are shown. The second approach to use silk as biomaterial is to dissolve silk by breaking up the molecular beta-sheet arrangement, prepare different arrangements of the silk and “regenerate” the structure by inducing beta-sheets again. Examples of hydrogels and the porous structure of a silk sponge are shown exemplarily.

These scaffolds are mainly used *in vitro* to generate tissue-like environments/niches for cells, especially stem cells to direct cellular processes in terms of proliferation and differentiation. For instance, in a recent project the encapsulation of chondrocytes in a silk-based hydrogel drastically increases the expression of cartilage-specific proteins like collagen type II or Aggrecan. This effect can even be observed for dedifferentiated chondrocytes, which is due to *in vitro* culture periods in the cartilage tissue-mimicking gels these cells start to re-differentiate and regain their chondrocytic phenotype and expression profile. We are working on the reproducible reactivation of dedifferentiated chondrocytes which (1) might be a cell-therapeutic option for cartilage lesions in future clinical applications and (2) might be an appropriate *in vitro* cartilage model.

Silk fibroin shows remarkable mechanical, degradation and biocompatability properties, favoring its use to generate highly loaded grafts, especially in the musculoskeletal field (A. H. Teuschl, Nürnberger, Redl, & Nau, 2013)(Nau & Teuschl, 2015). In this regard, the studies on a silk fibroin-based anterior cruciate ligament (ACL) graft (Hohlrieder et al., 2013; Andreas Herbert Teuschl, van Griensven, & Redl, 2014) and its recent evaluation in a large animal study will be presented. Prior to this study a protocol to thoroughly remove the contaminating sericin from the silk fibroin based textile engineered scaffold has been established. This method is based on simple boiling steps of the raw textile-engineered scaffold in an alkaline borate buffered solution (pH 9.0), which is dissolving the sericin under preservation of the underlying silk fibroin with its desirable mechanical characteristics (Andreas Herbert Teuschl et al., 2014). In our study, we were able to show that in contrast to our novel method, the classical method to remove sericin based on sodium carbonate solutions does not work with such hierarchically complex fibrous structures as our wire-rope designed scaffold. Scaffolds degummed in this way show mechanical properties similar to those of the native ACL tissue in terms of ultimative tensile strength as well as stiffness. For the testing of cell compatibility under mechanical straining a bioreactor system has been developed (Hohlrieder et al., 2013). In this system up to ten samples can be individually cultured at the same time and cyclically tensioned with defined longitudinal force for specific time patterns. In *pre-vivo* tests adipose tissue-derived stem cells have been cultured on the silk scaffolds and mechanically strained up to 3 weeks. These experiments showed that the cells stay viable on the scaffolds, colonize the whole scaffold under mechanical load and secrete a layer of ligament proteins (mostly collagen type I) on the silk fibroin fibers. After thorough *in vitro* testing, the material was also successfully applied *in vivo*. Since the first *in vivo* experiments in a rabbit model showed promising results in terms of excellent tissue compatibility and functionality also large animal studies in sheep have been performed. In these studies, the ACL of sheep has been replaced by the silk-based graft and the sheep have been observed for up to 12 months. After 12 months, the knee joints in all animals were stable and functional with no signs of damage to the surrounding knee structures such as menisci or hyaline cartilage which would occur in ACL-deficient knees. The silk-based graft had been completely invaded by cells from the adjacent tissues, which had partly degraded silk fibers and replaced them by ligament-like tissue. The results of this study have been recently accepted in the renowned *American Journal of Sports Medicine* (tentative publication date in June 2016).

In another study (Andreas Herbert Teuschl et al., 2015) we have created a textile-engineered so-called nerve guidance conduit also based on silk fibers. These types of scaffolds are intended to be used as guiding structures for regenerating peripheral nerves. The main tasks of these conduits should be acting as a barrier to invading fibroblasts from the surrounding tissue which might block nerve regeneration due to the formation of scar tissue, and the maintenance of a space for the regrowing nerves. For the generation of this structure, a protocol to fuse braided silk fibers to continuous layers has been developed. This process is based on the partial disintegration of silk fibroin molecules via the action of a ternary solvent consisting of $CaCl_2$, ethanol and water in a molar ratio of 1:2:8. Thorough *in vitro* testing revealed that the silk nerve guidance conduits created show excellent cell compatibility, tested via the use of primary Schwann cells, and mechanical properties for a possible *in vivo* use. Subsequent *in vivo* tests clearly demonstrated that the silk based nerve guidance conduit enables the reconnection of peripheral nerves in a so-called gap model.

In addition, functionalization strategies to enhance the cell-adhesive properties of silk-based grafts have been developed at the UAS Technikum Wien (Andreas H Teuschl et al., 2014) in order to enable the use of these grafts in “one-step surgical procedures”. The main drawback of tissue-engineering strategies involving cell-therapeutic steps is the time- and cost-intensive *ex vivo* culture of cells. Therefore current translational research aims to use cell sources where an already large number of cells can be extracted without the need for expanding cells *in vitro*. A promising candidate is fat tissue (gained by simple and low-invasive liposuction procedures) from which the stromal vascular fraction can be isolated containing endothelial cells and adipocytes alongside with a substantial portion of stem cells. Besides the cell issue, the seeding process of cells to an implant should be prompt and robust in order to enable the use of therapeutic cells in a tissue-engineering approach. In this regard, we developed a functionalization method of silk fibroin with plant lectins like wheat germ agglutinin (WGA), which leads to the mediation of cell adhesion in a time-frame (ofless than 20 mins) acceptable for a one-step surgical procedure. Plant lectins in general are carbohydrate-binding proteins, which are very specific for a certain sugar moiety. In the case of this study, WGA binds specifically to N-acetyl-D-glucosamine and sialic acid on the glycocalyx, sugar motifs that can be found on most mammalian cells such as stem cells. We were not only able to prove the drastic increase in cell-binding capacity of the functionalized silk but also that WGA-mediated binding increases the resistance of the cell-scaffold bonds against mechanical forces as well as to a proteolytic environment which is often associated with the inflamed implantation site of grafts. Moreover, we showed that the bound cells (adipose tissue derived stem cells) are still able to proliferate and to differentiate. In a recent publication (Guillaume, Park, Monforte, & Redl, 2016) the research by the UAS Technikum Wien in cooperation with colleagues from the Ludwig Boltzmann Institute for Clinical and Experimental Traumatology, a partner in the Austrian Cluster for Tissue Regeneration, could show that the technique of lectin-functionalization can improve the cell-binding properties of silk-based meshes intended to be used in abdominal wall hernia repair. Our data demonstrates that the modification of silk fibroin with WGA improves the cell-adhesion capacity of silk fibroin in a magnitude that enables cell-seeding in a one-step surgical procedure within an acceptable time frame (Andreas H Teuschl et al., 2014) for future clinical applications.

To further strengthen the expertise of the researchers of the UAS Technikum Wien,. Teuschl recently returned from a guest researcher stay in Prof. David Kaplan´s lab (Tufts University, Boston, USA), which is the pioneer in using silk as material in biomedical applications. This research stay was possible due to the Tissue Engineering International project funded by the City of Vienna (MA23, Project 14-06). In Kaplan´s lab, Teuschl started to work on some innovative ideas on the combination of silk fibroin with fibrin, the resulting polymerized network formed during blood clotting by the action of the protease thrombin on fibrinogen monomers. These new projects like all the those mentioned before are not only integrated into research at the Department of Biochemical Engineering but also into teaching practical courses for students.

In conclusion, silk fibroin has come a long way from ancient China and the Silk Road to today’s regard as a promising candidate for a wide range of applications in the field of Tissue Engineering and Regenerative Medicine.

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