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Regenerated silk materials for functionalized silk orthopedic devices by mimicking natural processing

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ABSTRACT

Silk fibers spun by silkworms and spiders exhibit exceptional mechanical properties with a unique combination of strength, extensibility and toughness. In contrast, the mechanical properties of regenerated silk materials can be tuned through control of the fabrication process. Here we introduce a biomimetic, all-aqueous process, to obtain bulk regenerated silk-based materials for the fabrication of functionalized orthopedic devices. The silk materials generated in the process replicate the nano-scale structure of natural silk fibers and possess excellent mechanical properties. The biomimetic materials demonstrate excellent machinability, providing a path towards the fabrication of a new family of resorbable orthopedic devices where organic solvents are avoided, thus allowing functionalization with bioactive molecules to promote bone remodeling and integration.

1. Introduction

The outstanding mechanical properties of natural silk fibers derive from their unique structure, which is determined by spinning process and the nature of the spinning solution. In the natural spinning process of silkworm, *Bombyx mori*, the silk fibroin (hereafter referred to as silk) concentration increases gradually and controllably from ~12% to 30% as the silk molecules move from the posterior to the anterior region of the silk glands [1]. Meanwhile, the chains assemble to micelles, follow by arrange and stack them together in a step-by-step manner, and form the compact solid architecture under regulation of external environments, such as pH, ion concentration, physical shear and/or elongational flow [2]. Many efforts have been explored to regenerate silk materials with mechanical properties comparable to or exceeding those of natural silk fibers [3–5]. Silk fibers stronger and tougher than natural undegummed *B. mori* silkworm silk have been obtained from regenerated *B. mori* silk solutions *via* microfluidic chip [3] and wet spinning [5]. One common feature of those studies is a spinning dope of highly concentrated silk solutions, which is a prerequisite to form a dense, compact solid structure in natural spinning process [1]. However, the focus has mainly been limited to the generation one-dimensional silk fibers in the micrometer range, which significantly hinders their application. There is need to develop three-dimensional silk materials in larger dimensions that can be used to fabricate devices with high mechanical demands, such as orthopedic devices.

Metals like titanium alloys and stainless steel remain the gold standard for orthopedic devices due to their robust mechanical properties and ease of fabrication and implantation, whereas limitations of stress shielding, infections, bone remodeling and second surgical removal have shifted significant interest to degradable devices [6,7]. Resorbable orthopedic devices composed of poly-L-lactic acid and polyglycolic acid reduces the need for hardware removal and improved bone remodeling. However, the degradation of these resorbable devices is associated with inflammatory foreign body reactions due to the acidic degradation products, osteolysis and incomplete bone remodeling [8]. In addition, orthopedic devices are historically designed to provide mechanical stability to the surrounding bone and soft tissue, whereas functionalization of the device to improve the implant integration and mitigate adverse events associated with the foreign body reaction or infection has

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been largely neglected [9]. Only recently, a few functionalization methods have been developed *via* applying biological coating to orthopedic devices to modulate the surrounding microenvironment. These approaches include coatings to enhance osteointegration (*e.g.*, calcium phosphate-like coatings [10–13]) and biomolecules coatings [14–18] and coatings to mitigate foreign body reaction (*e.g.*, bisphosphonates coatings [19–21]) and infection (*e.g.*, adhesion resistant coating [22–24], coatings with antibiotics [25,26] and silver impregnated coatings [27,28]). Surface coatings normally involve complicated procedures and they do not always possess long-term stability due to the degradation and detachment of the coatings. In contrast, bulk-incorporated bioactive molecules can exert their effects *via* both surface contact and sustained release. However, the bulk incorporation of labile biomolecules in current orthopedic devices remains a challenge due to the harsh conditions of the manufacturing process. Therefore, degradable orthopedic devices with appropriate mechanical properties, pro-osteogenic and antimicrobial features, would have a major impact on orthopedic repairs in promoting accelerated healing, reducing second surgeries and improving long-term patient outcomes.

Silk is an unique candidate to address these issues due to its excellent mechanical properties, biocompatibility, tunable degradability [29,30], as well as the ability of silk to stabilize various of bioactive compounds [31]. We recently demonstrated the feasibility of silk protein as a molding and machinable biomaterial system for the preparation of devices for craniofacial repairs, based on the use of an organic solvent-based process (1,1,1,3,3,3 hexafluro-2-propanol (HFIP)) [29]. To obtain water-stable structures, HFIP was required to facilitate silk solubility and methanol to induce the formation of β-sheet structure [29]. However, HFIP can limit utility due to cost, risk for residuals in the devices, and difficulty in incorporating labile biomolecules in the fabrication process. These caveats drive us to develop new types of silk orthopedic implants basing on the aqueous silk solution. However, high concentration aqueous silk solution (>20%, wt/wt), a prerequisite to form a dense, compact solid structure in natural silk spinning process [1], is highly instable and shows a strong tendency to form hydrogels or aggregates, which introduces challenges and increases complexity throughout the fabrication process. Natural spinning process of spiders and silkworms have inspired us to build bulk silk materials in a more facile and "green" way. In the present study, we developed a biomimetic process to generate bulk silk materials that avoided the use of organic solvents and harsh chemicals. The regenerated silk materials have excellent machinability and were successfully machined into various orthopedic devices. Importantly, the mild aqueous-based process facilitates the bulk incorporation of dopants and bioactive compounds, thus providing additional clinical benefits like osteoinductive, osteoconductive and anti-microbial/anti-inflammatory features.

2. Materials and methods

2.1. Preparation of aqueous silk solution

Silk fibroin solution was prepared from *B. mori* cocoons using our established protocol with some modifications [32]. First, sericin was removed by boiling the cocoon pieces in 0.02 M aqueous Na_2CO_3 solution for 30 min followed by extensive rinses in distilled water. The degummed silk was then dried overnight and dissolved in 9.3 M LiBr at 60 °C for 4 h, yielding a 20% (w/v) solution. The pH of LiBr solution was adjusted by adding 1 M LiOH solution so that the pH of the final silk solution after dialysis was 8.0. The silk/LiBr solution was dialyzed against distilled water for 2 days with 10 changes of water. The solution was then centrifuged for 2 × 20 min at 9000 rpm. The silk concentration was determined by evaporating water from a solution of known weight and weighing the remaining solid using an analytical balance.

2.2. P24 synthesis

The bone morphogenetic protein 2 (BMP2)-related peptide P24 (SKIPKASSVPTEL-SAISTLYLDDD) was synthesized by Tufts university core facility. The peptides were made on an ABI 431 Peptide Synthesizer using Fmoc chemistry and HBTU activation. The purity of the peptides was greater than 90%.

2.3. Preparation of silk-based blanks for machining

Silk solution of 6–8% (wt/wt) was subjected to forced airflow and water was slowly removed at 10 °C until the silk concentration reached 25–30%. The concentration of the solution was monitored by weighing the remaining solid after drying. Rectangular molds with water-permeable membranes were used to prepare silk blanks for machining. As an example, 3–12 ml Slide-A-Lyzer dialysis cassette (ThermoFisher Scientific, USA) with inner chamber dimensions of 65 mm × 28 mm × 6.5 mm was loaded with concentrated silk solution and placed into a refrigerated incubator with forced air flow at 10 °C for 3–4 days. Water evaporated through the porous water-permeable membrane from both sides of the cassette and resulted in solid silk materials. The materials were then left in a fume hood for 4 days followed by another 4 days in a 45 °C oven to remove the remaining water. To produce silk-based composite materials, silica (20–200 nm, Sigma-Aldrich, USA) and hydroxyapatite (HAP) (200 nm, Sigma-Aldrich, USA) were first dispersed in water by sonication and mixed with silk fibroin solution to obtain a suspension with desired amount of silica or HAP. The same dehydration procedure as described above was conducted to obtain silk/SiO_2 and silk/HAP composite blanks. To produce antibiotic-containing silk materials, a suspension of ciprofloxacin•HCl in water was mixed with 26.5% (wt/wt) silk solution to a final ciprofloxacin content of 5% (wt/wt). The mixture was loaded into molds and dehydrated at 10 °C for 3 days followed by room temperature drying in a fume hood for 2 weeks. Osteoinductive silk materials were obtained in a similar way except that BMP2 (Wyeth, USA) and P24 solution was mixed with concentrated silk solution at a concentration of 30 μg BMP2/g silk and 1.0 mg P24/g silk, respectively. The pH of silk solution used for BMP2 mixing was maintained at 6.5 to prevent the aggregation of BMP2 at pH 8, as BMP2 has an isoelectric point of 8.5 [15].

2.4. Machining of silk orthopedic devices

The silk blanks were machined into screws and intramedullary (IM) nails using a CNC lathe (Trak TRL 1440 EX, Southwestern Industries, USA). For IM nails, the silk blank was left on the CNC lather once the desired diameter was reached and a needle type tip was placed on the end for insertion. For screws with bone screw threads, a custom single point external cutter (Vargus, USA) was used on the CNC lathe to cut screw threads by matching turning speed with horizontal speed of the cutter to cut a desired pitch length (outer diameter ~ 1.8 mm, pitch = 600 μm). The screw heads were machined to have a cylindrical heads by use of the CNC lathe. Once machined, the screw or IM nail was then cut off behind the head or length of nail. A diamond cutter was mounted to the lathe and used to cut a slot in the screw head for screw insertion. For plates, rectangular silk blanks were machined using a CNC milling machine (Trak DPM, Southwestern Industries, USA). The milling machine was used to cut the desired shape of the mold and thickness (Supplementary Fig. S6). Once the plate was shaped and