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Injectable silk-polyethylene glycol hydrogels

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ABSTRACT

Silk hydrogels for tissue repair are usually pre-formed via chemical or physical treatments from silk solutions. For many medical applications, it is desirable to utilize injectable silk hydrogels at high concentrations (>8%) to avoid surgical implantation and to achieve slow in vivo degradation of the gel. In the present study, injectable silk solutions that formed hydrogels in situ were generated by mixing silk with low-molecular-weight polyethylene glycol (PEG), especially PEG300 and 400 (molecular weight 300 and 400 g mol^{-1}). Gelation time was dependent on the concentration and molecular weight of PEG. When the concentration of PEG in the gel reached 40–45%, gelation time was less than 30 min, as revealed by measurements of optical density and rheological studies, with kinetics of PEG400 faster than PEG300. Gelation was accompanied by structural changes in silk, leading to the conversion from random coil in solution to crystalline β-sheets in the gels, based on circular dichroism, attenuated total reflection Fourier transform infrared spectroscopy and X-ray diffraction. The modulus (127.5 kPa) and yield strength (11.5 kPa) determined were comparable to those of sonication-induced hydrogels at the same concentrations of silk. The time-dependent injectability of 15% PEG–silk hydrogel through 27 G needles showed a gradual increase of compression forces from ~10 to 50 N within 60 min. The growth of human mesenchymal stem cells on the PEG–silk hydrogels was hindered, likely due to the presence of PEG, which grew after a 5 day delay, presumably while the PEG solubilized away from the gel. When 5% PEG–silk hydrogel was subcutaneously injected in rats, significant degradation and tissue in-growth took place after 20 days, as revealed by ultrasound imaging and histological analysis. No significant inflammation around the gel was observed. The features of injectability, slow degradation and low initial cell attachment suggests that these PEG–silk hydrogels are of interest for many biomedical applications, such as anti-fouling and anti-adhesion.

1. Introduction

Silk fibroin is the major structural protein in silkworm cocoons, and can be isolated from the immunogenic coatings through an alkaline boiling process called degumming [1,2]. The degummed silk fibroin fibers, characterized with a high content of crystalline β-sheet structure, can be dissolved in protein denaturants such as 9.3 M lithium bromide, generating silk fibroin protein solutions with random coil-dominated structures. A silk fibroin solution free of salts can be obtained after removal of the denaturants by dialysis. This purified silk solution can be used to prepare a variety of biomaterials, e.g. films, hydrogels, nano- and microspheres, nanofibers and sponges, via all-aqueous processing methods that induce a structural transition back to β-sheets via molecular self-assembly due to enhanced intermolecular interactions [2,3]. Silk biomaterials, due to their desirable mechanical strength, biocompatibility, degradation and controlled drug release, have been widely used for tissue engineering and drug delivery. Silk hydrogels, unlike many other polymeric hydrogels, can be prepared via physical treatments of the silk fibroin solution, such as via sonication, vortexing and electrical fields [4–6]. Since no harmful solvents or compounds are used in these processes, the silk hydrogel is a useful carrier for encapsulating and delivering cells as well as bioactive molecules, towards tissue repairs or therapeutics. Pre-formed silk hydrogels prepared by low pH or sonication have been used to repair bone defects in previous studies [7–9].

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Encapsulation and proliferation of human mesenchymal stem cells (hMSCs) in silk hydrogels was achieved over 3 weeks when the gel concentration was lower than 4% (w/v) [4]. In a related study, immature chondrocytes encapsulated in sonication-induced silk hydrogels were used for cartilage tissue engineering [10]. Silk hydrogel was also used to deliver monoclonal antibodies. The antibody-encapsulated silk gels were lyophilized, and demonstrated long term sustained release of bioactive antibodies after rehydration [11]. In all these studies, silk hydrogels were pre-formed prior to in vitro testing or surgical implantations. For many applications where minimally invasive administration is required, e.g. ophthalmology, otolaryngology and cardiology, injections of silk hydrogels through a thin needle would be desirable, and the concentration of silk hydrogel should be higher than 8% (w/v) in order to achieve in vivo degradation longer than 3 months ([8] and unpublished data). Unfortunately, due to the extensive and strong silk fiber network, silk hydrogels with a concentration above 8% (w/v) are difficult to inject through a thin needle (>25 G).

To address these limitations, one option would be to inject high concentration of silk solution and trigger silk gelation in situ within minutes. For many synthetic polymers, in situ formed hydrogels can be achieved by applying external stimuli, e.g. light, ultrasound and shear, to the solution post-injection. This strategy, however, cannot be easily used for naturally derived biopolymers without chemical modification to link specific reactive groups on the polymer chains. Silk fibroin forms hydrogels when the solution was mixed with acids, salts, alcohols, surfactants and polymers [12–15]. However, toxicity of these chemicals is a significant concern for medical applications. To achieve rapid gelation within 30 min, greater than 50% alcohol and 1% sodium dodecyl sulfate (SDS) usually needs to be added to silk solution, which is above the tolerance level (<10% alcohol for intravenous injection and <1% SDS for skin and ocular formulations) for human use [16,17]. These chemical mixing methods have therefore been restricted to silk gelation mechanisms or tissue repairs using gel implants after extraction of these chemicals. Physical methods, such as sonication and vortexing, to induce silk gelation are non-toxic. Depending on the energy applied to the silk solution, gelation can take place within a few minutes, which is ideal for encapsulating and delivering cells for tissue regeneration [4]. However, control of gelation time depends on the concentration of the silk solution and the instrumental parameters (sonication energy output, time, silk, position of sonication probe), which can be empirical. Furthermore, sterilization is another obstacle for using these methods to induce silk gelation in situ, as the sonicator probe to be inserted into the silk solution needs to be kept sterile, which is difficult in most clinical environments. Thus, developing a safe, simple, controllable and practical method to enable high concentration silk hydrogel injection would be a significant advance and is of importance for biomedical applications.

In this study, we explored the use of low-molecular-weight, liquid-state polyethylene glycol (PEG) to induce silk gelation. Gelation kinetics and gel properties were investigated using spectroscopic, mechanical and biological approaches, with an aim to identify optimal conditions for high concentration gel injections. PEG is a nontoxic, water-soluble, bioinert synthetic polymer that is widely used in pharmaceutical industries. PEG300 and PEG400 (molecular wight 300 and 400 g mol^{-1}, respectively) are commercially available and FDA-approved excipients for oral, topical and parenteral drug administration [16]. The final concentration of PEG300 and 400 can go up to 50% in the formulations for intravenous and intramuscular injection without any toxic effects [16]. High-molecular-weight PEGs have been previously used to concentrate silk solution and aid in making porous scaffolds [18]. In a previous study, PEG600 was blended with silk fibroin solution with an aim to prepare porous scaffolds for tissue engineering. Gelation time and silk structural changes were characterized in these studies [19,20]. Our group has recently developed a silk–PEG hydrogel technique based on chemical crosslinking between two reactive PEG components, and silk was entrapped in the PEG gel network, providing mechanical strength and slow degradation [21].

2. Materials and methods

2.1. Materials

Partially degummed silk fibers were purchased from Xiehe Silk Corporation (Shengzhou, Zhejiang province, China). Pharmaceutical grade PEG, molecular weight (MW) 300 g mol^{-1} (Kollisolv® PEG E300, catalog number: 91462, average MW 285–315 g mol^{-1}), PEG MW 400 g mol^{-1} (Kollisolv® PEG E400, catalog number: 06855, average MW 380–420 g mol^{-1}), recombinant human insulin (catalog number: I2643), human insulin ELISA kit (catalog number: RAB0327) and other chemical reagents were all purchased from Sigma-Aldrich (St Louis, MO). Cell culture medium and other reagents were purchased from Life Technologies (Grand Island, NY). Wistar rats weighing 200–300 g were provided by the Animal Research Center, the Second Hospital of Harbin Medical University, China.

2.2. Silk purification

To remove residual sericin contaminants, the silk fibers purchased were further degummed in the lab following the procedures in the literature [22]. Briefly, silk fibers were boiled in 0.02 M sodium carbonate solution for 30 min, rinsed with ultrapure water three times, drained and dried in a fume hood overnight. The dried fibers were dissolved in 9.3 M lithium bromide solution at 60 °C to obtain a concentration of 20%. The solution was dialyzed against pure water for 2 days to remove the lithium bromide and centrifuged to remove insoluble fibrous debris. The concentration of purified silk was ~6% (w/v). The solution obtained was autoclaved and stored at 4 °C. To prepare low concentration silk hydrogels, silk solution was diluted with water; and to prepare high concentrations, the solution was freeze-dried using a lyophilizer (CHRIST Alpha 2-4 LSCplus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) and reconstituted with water following the procedure in the literature [23].

2.3. PEG–silk hydrogel preparation

PEG solution with a defined weight percentage (w/w) was prepared by mixing liquid state PEG, MW 300 g mol^{-1} (PEG 300) or MW 400 g mol^{-1} (PEG400) with ultrapure water. After mixing and filtration through a 0.22 μm filter, the PEG solutions were stored at room temperature. To make PEG–silk hydrogels, PEG was mixed with silk in a glass vial at a volume ratio of 1:1. The vial was gently mixed and the solution was subjected to analyses or loaded into syringes for injection tests. For cell culture and in vivo studies, silk solution was autoclaved prior to the mixing. Autoclaving did not significantly change the gelation time and gel properties, similar to our previous report [4].

2.4. Characterization

2.4.1. Optical density (OD) measurement

Gelation kinetics were determined by following OD changes of the PEG–silk mixture, as reported previously [12]. Following the addition of silk and PEG, the mixture was aliquoted into a 24-well plate, with 200 μl per well. The plate was subjected to OD measurements at 550 nm under the kinetics mode on a UV–visible

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