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Scale-up of water-based spider silk film casting using a film applicator

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ABSTRACT

Spider silk proteins for applications in drug delivery have attracted an increased interest during the past years. Some possible future medical applications for this biocompatible and biodegradable material are scaffolds for tissue engineering, implantable drug delivery systems and coatings for implants. Recently, we reported on the preparation of water-based spider silk films for drug delivery applications. In the current study, we describe the development of a manufacturing technique for casting larger spider silk films from aqueous solution employing a film applicator. Films were characterized in terms of morphology, water solubility, protein secondary structure, thermal stability, and mechanical properties. Different post-treatments were evaluated (phosphate ions, ethanol, steam sterilization and water vapor) to increase the content of β-sheets thereby achieving water insolubility of the films. Finally, the mechanical properties of the spider silk films were improved by incorporating 2-pyrrolidone as plasticizer.

1. Introduction

Spider silk has been used for medical purposes since ancient history. For instance, it was applied in Roman and medieval dermatological practices to facilitate wound healing (Newman and Newman, 1995). In the modern ages the use of this outstanding material, famous for its excellent mechanical properties (Schacht and Scheibel, 2014; Brown et al., 2011; Spiess et al., 2010a), has at first been limited to research purposes. Product development has been disregarded due to the many difficulties encountered in farming spiders (Doblhofer et al., 2015; Hardy and Scheibel, 2010). The large scale production of recombinant spider silk proteins in *Escherichia coli* (Huemmerich et al., 2004; Schmidt et al., 2007; Heidebrecht and Scheibel, 2013) opened a new range of possibilities, including the opportunity to specifically modify the primary structure of these biopolymers and to design new proteins with an enlarged functionality portfolio (Schacht and Scheibel, 2014). Coatings made of micro scale spider silk films have been shown to be an excellent bioshield. In fact, in a recent work by Zeplin et al., eADF4(C16) coatings were able to reduce post-operative inflammation after inserting medical grade silicone implants in animals (Zeplin et al., 2014). As a consequence of inhibiting fibroblast proliferation and the synthesis of collagen I, the capsular fibrosis formation was reduced. Furthermore, spider silk films have been shown to be promising implantable drug delivery matrices, both for small molecules (Hardy et al., 2013) and for proteins (Agostini et al., 2015). Spider silk matrices can be produced through a large number of different techniques as recently reported by Borkner et al. (Borkner et al., 2014). The list of technologies that can be employed include: dip or spin coating (Zeplin et al., 2014; Junghans et al., 2006; Spiess et al., 2010b; Greving et al., 2012), layer-by-layer deposition (Decher, 1997), Langmuir-Blodgett deposition (Zasadzinski et al., 1994; Agarwal, 1988), electrospinning (Greiner and Wendorff, 2007; Zhang et al., 2012, 2009; Zhu et al., 2007; Zhou et al., 2008; Park et al., 2004), electrophoretic deposition (Boccaccini et al., 2010; Zhang et al., 2011; Maniglio et al., 2010), and finally lithography (del Campo and Arzt, 2008; Tawfick et al., 2012). However, the most adopted method to cast spider silk films remains the solvent evaporation technique (Schacht and Scheibel, 2014; Hardy et al., 2013; Agostini et al., 2015; Spiess et al., 2011; Metwalli et al., 2007; Slotta et al., 2006; Müller-Herrmann and Scheibel, 2015). Since this technology is already integrated in cast film lines in industry, it is in principle easy to adapt from lab-scale to industrial production. But so far, the solvent evaporation technique for spider silk films has only been used in lab scale by direct casting into PTFE forms. Challenges of this manual process are the variation in films thickness and the control of film geometry. Additionally, the number of films produced per time is very limited. Therefore, this work was dedicated to the improvement this manufacturing technique by using an automatic film applicator.

2. Materials

The spider silk protein eADF4(C16) was kindly provided by AMSilk GmbH (Martinsried, Germany). Guanidinium thiocyanate (GdmSCN), trizma® base (Tris), 2-pyrrolidone, as well as polyethylene glycol (PEG) 20 kDa were purchased from Sigma-Aldrich (Steinheim, Germany).

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3. Methods

3.1. Film production

Spider silk proteins were dissolved in 6 M guanidinium thiocyanate and subsequently dialyzed against 5 mM Tris/HCl buffer, pH 8 at 4 °C. The pH of the protein solution was determined using a pH meter MP 220 (Mettler Toledo, Giessen, Germany). The protein solution was centrifuged at 4 °C for 15 min at 10,000 rpm and subsequently filtered through a 0.45 µm cellulose acetate filter. Afterwards, the spider silk solution was dialyzed against 5 mM Tris/HCl buffer, pH 8 at 4 °C, containing 10% w/v of PEG 20,000 Da. Protein concentration was determined photometrically using a NanoDrop 2000 system from peqlab (Erlangen, Germany). The protein concentration of the casting solution was finally adjusted to 5% w/v adding 5 mM Tris/HCl buffer. Spider silk films were cast on a plastic foil A5 22/5 B from mtv-messtechnik (Koeln, Germany) at room temperature using a film applicator Coatmaster 510 (Erichsen, Hemer, Germany) equipped with a casting knife of 2000 µm. Casting was performed at a velocity of 1 mm/s. 2 mL of the casting solution were used to cast each film. The surface coverage was about 100 cm^2. The film applicator was placed in a laminar flow cabinet (Thermo Scientific, Munich, Germany). After preparation, films were dried overnight at room temperature in the laminar flow cabinet which was closed and switched off. Finally, films were cut into samples of 2.5 × 3 cm using a scalpel. Films containing 2-pyrrolidone were prepared dissolving 2% w/v of the plasticizer directly in the casting solution.

3.2. Spider silk film morphology

Photographs of eADF4(C16) films were obtained using a digital camera DSC-S75 (Sony Corporation, Tokyo, Japan). Scanning electron micrographs of the film surface were collected after the films were immobilized on Leit-Tabs (Plano GmbH, Wetzlar, Germany) to a sample holder. Samples were carbon sputtered under vacuum and analyzed using a Joel JSM-6500F field emission scanning electron microscope (Joel Inc., Peabody, USA).

3.3. Thermal analysis

Thermograms of spider silk films were obtained by differential scanning calorimetry (DCS 204 Netzsch, Selb, Germany). Spider silk films were loaded in aluminum pans and a small hole was punched in the pan covers. Samples were heated under nitrogen flow at 10 K/min up to 110 °C, then cooled to −40 °C, followed by heating to 400 °C as described previously (Spiess et al., 2011).

3.4. Protein secondary structure

Fourier transform infrared (FT-IR) spectra were collected using the Hyperon microscope from Bruker Optik (Bruker, Germany) using a 20× attenuated total reflectance objective (ATR). The spectra were an average of 120 scans at the resolution of 4 cm^{-1}. All measurements were performed in the range of 600 and 4000 cm^{-1}. Films were measured in three different areas: in the middle, next to the edge and between the middle and the edge.

3.5. Dissolution of spider silk protein from cast films in water

Films were weighed using a balance AT261 Delta Range from Mettler Toledo (Giessen, Germany) and placed in a 6-well plate. Each film was covered with 2 mL of highly purified water (HPW). The well plate was positioned on a shaking plate at 2 rpm at room temperature. After 1 h, the water was removed and analyzed using a spectrophotometer (Agilent 8453, Boeblingen, Germany). Protein concentration in solution was determined photometrically. The amount of the dissolved spider silk was compared with the initial mass of the film obtaining the percentage of the loss in mass of the film in water.

3.6. Post-treatments

3.6.1. PO_4^{3-} post treatment

Films were post-treated by spraying a 2 M phosphate solution on the film surface using an ultrasonic nozzle Sonotek 120-00456 (Sonotek, Milton, USA) at a flow rate of 1 mL/min at 120 kHz for10 s. Post-treated films were left to dry at room temperature under laminar flow.

3.6.2. Ethanol post-treatment

Films were post-treated by spraying a 70% ethanol-water solution on the film surface using an ultrasonic nozzle Sonotek 120-00456 (Sonotek, Milton, USA) as described above. Post-treated films were left to dry at room temperature under laminar flow.

3.6.3. Steam sterilization

Spider silk films were steam sterilized using an autoclave GTASO (Fritz Goessner GmbH & Co, Hamburg, Germany). Briefly, air was saturated with water vapor, then the temperature was raised to 121 °C, 1 bar, held for 15 min, and finally the system was cooled down to room temperature.

3.6.4. Water vapor treatment

Spider silk films were sealed in autoclave bags and stored for 60 min in a desiccator (Fig. 1). The desiccator's reservoir was filled with highly purified water preheated at 60 °C. The temperature was monitored using a digital temperature and humidity sensor (TFA Dostmann, Wertheim-Reicholzheim, Germany).

3.7. Mechanical properties

Films were cut into samples of 2 × 120 mm. The thickness was determined using a Mitutoyo pen (Mitutoyo Deutschland GmbH, Neuss, Germany). Tensile test of spider silk films (n = 5) were carried out at 24 °C and 32% RH; spider silk films containing 1% w/v 2-pyrrolidone (n = 5) were analyzed at the same conditions. All spider silk films were tested in a dry state. Measurements were performed using a Zwick tensile tester Z0.5 (Zwick GmbH & Co. KG, Ulm, Germany), equipped with a 5 N capacity load cell.

4. Results and discussion

4.1. The scale up process

The film applicator used in this work is controlled by a

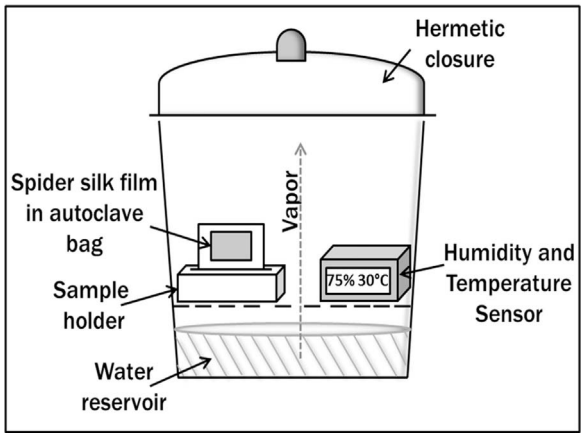

Fig 1. Water vapor treatment set up.