Biomaterials 35 (2014) 8613–8620

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Silk fibroin rods for sustained delivery of breast cancer therapeutics

Tuna Yucel, Michael L. Lovett, Raffaella Giangregorio, Erin Coonahan, David L. Kaplan*

Tufts University, Biomedical Engineering, Medford, MA 02155, USA

ARTICLE INFO

Article history:
Received 10 June 2014
Accepted 15 June 2014
Available online 6 July 2014

Keywords:
Small molecule drugs
Anastrozole
Biocompatibility
Biodegradation
Mechanisms
Modeling

ABSTRACT

A silk-protein based reservoir rod was developed for zero-order and long-term sustained drug delivery applications. Silk reservoir rod formulations were processed in three steps. First, a regenerated silk fibroin solution, rich in random-coil content was transformed into a tubular silk film with controllable dimensions, uniform film morphology and a structure rich in silk *II*, β-sheet content via "film-spinning." Second, the drug powder was loaded into swollen silk tubes followed by tube end clamping. Last, clamped silk tube ends were sealed completely via dip coating. Anastrozole, an FDA approved active ingredient for the treatment of breast cancer, was used as a model drug to investigate viability of the silk reservoir rod technology for sustained drug delivery. The *in vitro* and *in vivo* pharmacokinetic data (in a female Sprague–Dawley rat model) analyzed via liquid chromatography-tandem mass spectroscopy indicated zero-order release for 91 days. Both *in vitro* and *in vivo* anastrozole release rates could be controlled simply by varying silk rod dimensions. The swelling behavior of silk films and zero-order anastrozole release kinetics indicated practically immediate film hydration and formation of a linear anastrozole concentration gradient along the silk film thickness. The dependence of anastrozole release rate on the overall silk rod dimensions was in good agreement with an essentially diffusion-controlled sustained release from a reservoir cylindrical geometry. *In vivo* results highlighted a strong *in vitro-in vivo* pharmacokinetic correlation and a desirable biocompatibility profile of silk reservoir rods. During a 6-month implantation in rats, the apparent silk molecular weight values decreased gradually, while rod dry mass and β-sheet crystal content values remained essentially constant, providing a suitable time-frame for controlled, long-term sustained delivery applications. Overall, the silk reservoir rod may be a viable candidate for sustained delivery of breast cancer therapeutics.

1. Introduction

Sustained delivery therapeutics generally aim to reduce the dosing frequency of chronic-use medications, while maintaining essentially constant therapeutic plasma concentrations. The potential clinical benefits of sustained drug delivery include reduced side effects and improved patient compliance, along with lower costs for third-party payers. Polylactide-co-glycolide acid (PLGA) synthetic polymer based products currently dominate the sustained delivery market [1]. Despite their desirable pharmacological and hydrolytic biodegradation characteristics, PLGA systems also have inherent limitations that may lead to drug instability and safety concerns [2–5]. In efforts to mitigate these issues, significant research has been devoted to silk protein-based sustained delivery therapeutics [6–9]. The growing interest in silk protein is mainly due to its rare combination of desirable properties, including biocompatibility and controllable surface biodegradation into non-inflammatory by-products, aqueous purification and processing capabilities, compatibility with terminal sterilization, robust mechanical properties without chemical cross-linkers, and its utility as a drug stabilization matrix [10–23].

In Nature, the largest silk producer is the domestic silkworm, *Bombyx mori*. The silk fibroin heavy chain, the structural protein component of *B. mori* silk is a high molecular weight ($\approx$350 kDa) block copolymer, essentially consisting of hydrophobic β-sheet crystallizable domains interspersed with amorphous spacers, with an overall anionic character ($pI_{fibroin} \approx 4$) [24]. Silk fibroin displays a predominantly hydrophobic sequence that results in strong intramolecular and intermolecular physical interactions, stimuli-responsive self-assembly pathways, along with a crystal polymorphism that can be manipulated through processing conditions [25–30]. This structural versatility of silk fibroin facilitated the development of a sustained drug delivery toolkit over the past

* Corresponding author. Tufts University, Department of Biomedical Engineering, 4 Colby Street, Medford, MA 02155, USA. Tel./fax: +1 617 627 3251.
E-mail address: david.kaplan@tufts.edu (D.L. Kaplan).

http://dx.doi.org/10.1016/j.biomaterials.2014.06.030
0142-9612/

decade, including micro/nanoparticle suspensions, bioadhesives, injectable hydrogels, and implantable scaffolds, films, tubes and rods [6–9,14,29–38]. For small molecules, the published reports on silk-based sustained delivery systems mainly consist of implants with promising pharmacokinetic profiles [39,40]. For example, *in vitro* near zero-order sustained release duration was 14 and 31 days for silk fibroin-adenosine tablets and silk fibroin-erythromycin porous sponges, respectively [39,40]. However, from a patient compliance perspective the critical implant dimensions should be minimized, and the minimum inter-dosing duration required to justify a potentially invasive administration procedure should be considered. Furthermore, essential first steps to demonstrate proof of concept for a silk-based implant sustained delivery technology should include formulation and analytical development to achieve controllable and well-characterized physicochemical properties, along with *in vivo* pharmacokinetic assays to ensure sustained delivery at therapeutic levels.

To that end, we previously reported a process for the preparation of silk fibroin reservoir rods with controlled morphology, structure and overall dimensions [32]. Briefly, this method involves the preparation of silk fibroin tubes using "film-spinning" followed by drug loading and tube end capping. In film-spinning, a high concentration silk fibroin solution is injected onto a rotating mandrel at a strictly controlled flow rate and is immediately exposed to a critical heat-treatment step using an in-line heating element. The film-spinning process results in a tubular silk fibroin film with uniform and controlled thickness, and a predominantly silk *II*, β-sheet crystal structure. A tight control over the dimensions and structure of silk fibroin tubes may be essential for sustained drug delivery applications. After film spinning, the silk film tubes are hydrated and loaded with a desired therapeutic. Finally, silk fibroin tube ends are dip coated to ensure a complete seal and prevent dose dumping. Here, we demonstrate zero-order, long-term sustained release of a model breast cancer drug from silk fibroin reservoir rods.

Breast cancer, i.e., ductal and lobular carcinomas currently encompasses over 20% of all cancer cases in women worldwide. One of the treatment options for hormone receptor positive disease is the use of non-steroidal aromatase inhibitors, such as ARIMIDEX™. Anastrozole, the active ingredient of ARIMIDEX™, is a potent (1 mg p.o. q.d.) small molecule drug ($C_{17}H_{19}N_5$, m = 293.4 g/mol) with moderate water solubility (0.5 mg/ml at 25 °C), moderate lipophilicity (log P(octanol/water) = 1.58), and non-ionic character at neutral pH (pKa = 1.4) [41]. However, there is growing concern about patient adherence to aromatase inhibitor therapy [42] and large reported differences in patient self-report on adherence and actual medication delivery results even within the first year [43]. We believe one possible means to improve patient compliance would be through zero-order, sustained anastrozole delivery from silk fibroin reservoir rods.

2. Materials and methods

2.1. Materials

Degummed silk fibroin fibers were purchased from Suho Biomaterials Technology (Suzhou, China). Anastrozole, chlorpheniramine and all other chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Regenerated silk fibroin solution

A 20 wt.% solution of degummed silk fibroin fibers in 9.3 M aqueous LiBr was dialyzed against deionized water ($\rho \approx 18.2$ MΩ cm) for 48 h using Slide-A-Lyzer dialysis cassettes (3 kDa MWCO, Fisher Scientific, Pittsburgh, PA). The conductivity of the dialysis water was probed to ensure completion of desalting. The final concentration of the regenerated silk solution was 7 ± 1 wt.%. Silk solution resistivity, pH and high shear viscosity (5 wt.% silk) values were 25 ± 5 kΩ cm, 8.5 ± 0.5 pHU and 3.1 ± 0.5 cP at 25 °C, respectively (mean ± SD, $n = 3$). An apparent molecular weight distribution was characterized via size exclusion chromatography (SEC). One microgram of silk protein was injected into an analytical column (SEC-3, 4.6 mm × 300 mm, 300 Å, Agilent, Santa Clara, CA) using an Agilent 1200 Series HPLC pump and 1× PBS with 0.05 wt.% NaN_3 as the mobile phase. The molecular weight standards were cytidine (243 Da), bovine serum albumin (67 kDa), γ-globulin (158 kDa) and thyroglobulin (660 kDa). The calculated apparent weight averaged molecular weight (M_W) values were 198 ± 15 kDa (mean ± SD, $n = 3$). Silk fibroin solution was concentrated to 28–35 wt.% via dialysis against 15–20 wt.% aqueous PEG (10 kDa) for 16–24 h using 3 kDa MWCO Slide-A-Lyzer dialysis cassettes. Silk concentration was measured gravimetrically and via Bradford Assay to within ±0.5 wt.%.

2.3. Silk-anastrozole reservoir rods

A custom setup was developed for film-spinning silk fibroin tubes as depicted in Fig. 1. Briefly, concentrated silk fibroin solution (28–35 wt.%) was injected at a flow rate between 0.15 and 0.50 mm^3/s through a narrow gauge needle (≥21 G) onto a PTFE-coated stainless steel wire (McMaster-Carr, Atlanta, GA). The injection rate was controlled using a syringe pump (KD Scientific, Holliston, MA). During injection, the wire was concomitantly reciprocated horizontally at 0.33 mm/s, while being rotated along its axis at 1 Hz. The motion of the wire was controlled through an AC gear motor (McMaster-Carr, Atlanta, GA) connected to another syringe pump (KD Scientific, Holliston, MA). Immediately after injection of silk fibroin solution, the rotating wire was transferred into a tube oven to heat-treat the silk fibroin solution, typically at 80 ± 5 °C for 300 s to obtain a 0.05–0.10 mm thick film. The simultaneous

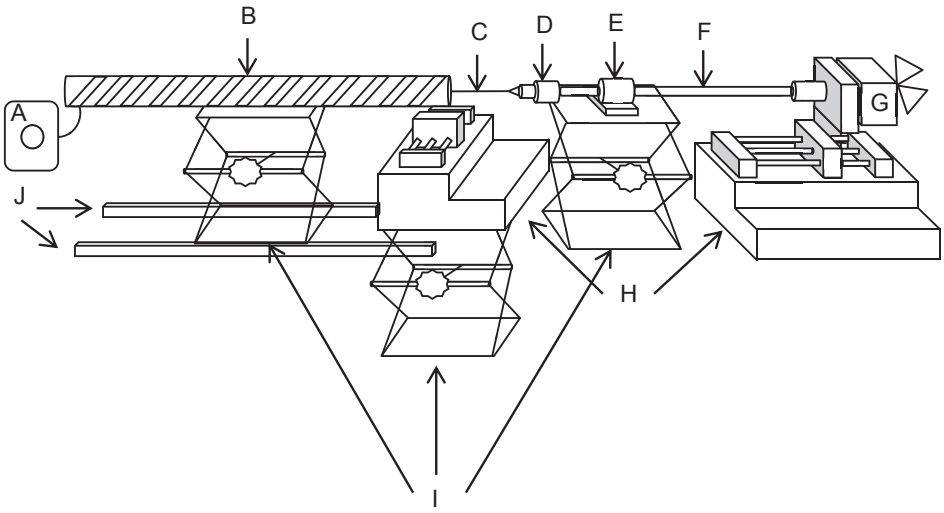

Fig. 1. A schematic representation of the silk film-spinning setup consisting of A: Temperature controller; B: Tube oven; C: PTFE/SS wire; D: Drill chuck; E: Bearing; F: Shaft: G: AC gear motor; H: Syringe pumps; I: Adjustable height stands; J: Slides.