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Lyophilized silk fibroin hydrogels for the sustained local delivery of therapeutic monoclonal antibodies

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ABSTRACT

The development of sustained delivery systems compatible with protein therapeutics continues to be a significant unmet need. A lyophilized silk fibroin hydrogel matrix (lyogel) for the sustained release of pharmaceutically relevant monoclonal antibodies is described. Sonication of silk fibroin prior to antibody incorporation avoids exposing the antibody to the sol–gel transition inducing shear stress. Fourier Transform Infrared (FTIR) analysis showed no change in silk structural composition between hydrogel and lyogel or with increasing silk fibroin concentration. Antibody release from hydrogels occurred rapidly over 10 days regardless of silk concentration. Upon lyophilization, sustained antibody release was observed over 38 days from lyogels containing 6.2% (w/w) silk fibroin and above. In 3.2% (w/w) silk lyogels, antibody release was comparable to hydrogels. Swelling properties of lyogels followed a similar threshold behavior. Lyogels at 3.2% (w/w) silk recovered approximately 90% of their fluid mass upon rehydration, while approximately 50% fluid recovery was observed at 6.2% (w/w) silk and above. Antibody release was primarily governed by hydrophobic/hydrophilic silk-antibody interactions and secondarily altered by the hydration resistance of the lyogel. Hydration resistance was controlled by altering β-sheet (crystalline) density of the matrix. The antibody released from lyogels maintained biological activity. Silk lyogels offer an advantage as a delivery matrix over other hydrogel materials for the slow release of the loaded protein, making lyogels suitable for long-term sustained release applications.

1. Introduction

The medical importance of monoclonal antibody therapeutics continues to grow. Over 300 such therapeutics are under development and more than 20 are already approved [1]. Antibody based therapies are being developed for a wide range of indications in oncology, immune mediated disorders and wound healing [1,2]. Many of these indications require repetitive dosing lasting anywhere from several weeks to months, and sometimes for the lifetime of the patient [2]. Patient compliance and drug efficacy would be maximized by the development of long-term sustained or localized delivery therapies [3]. Despite these advantages, most protein therapeutics are developed for either intravenous (IV), intramuscular (IM), or subcutaneous (SubQ) administration with bolus dosing. Recombinant human bone morphogenetic protein-2 (rhBMP-2) with a collagen sponge is the only approved implantable protein–matrix combination therapy for local delivery [4,5]. The challenges in manufacturing inherently unstable protein therapeutics are exaggerated if a combination therapy is being developed [6–8]. The availability of versatile and biocompatible sustained delivery matrices that maximize therapeutic protein stability continues to be a significant unmet need.

Biodegradable polymers have been most intensely investigated as possible matrices for sustained release of proteins. The majority of studies have been performed on two types of delivery strategies: micro/nano-spheres and hydrogel-based matrices [9–15]. Both types of matrices have been engineered using synthetic and natural polymers, with the most commonly used synthetic polymers being poly-(DL-lactide-co-glycolide) (PLGA), and poly(vinyl alcohol) (PVA) [9,16,17]. The material properties of synthetic polymers can be customized by controlling the composition of monomers, altering polymerization conditions, or introducing functional groups [18]. Maintaining protein stability during encapsulation in these synthetic polymers has been problematic due to significant differences in hydrophobicity and unfavorable microclimates caused by degradation products [6,7,18,19]. Natural polymers that have been studied for protein release include collagen, gelatin, fibrin, hyaluronic acid, chitosan and alginate [20]. Natural polymers have the

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advantage of typically being biocompatible and biodegradable, but batch to batch variability and a more limited range of physical properties can be restrictive [20].

Tolerance of hydrogels by living tissue and compatibility with bioactive agents such as cells and proteins have contributed to their popularity as biomaterials [13,21,22]. Hydrogels are created by the cross-linking of polymer chains, leading to the creation of a three dimensional, structurally integral, hydrophilic matrix [8,13]. Hydrogel constructs have been used for protein delivery as is, in the hydrated state, or they have been dehydrated through air drying or lyophilization, and subsequently rehydrated for use [5,23,24]. Sustained release from hydrogels is characterized by a rate-limiting step which can be diffusion-based or swelling-based [8]. Diffusion of drugs is not typically retarded to a significant degree in the swollen state of a hydrogel, as the typical mesh sizes for hydrogels range from 5 to 100 nm [8]. Therefore, hydrophilic and rapidly swelling hydrogels are limited in sustained release capabilities, making them suitable for short-term but not mid- or long-term release needs.

Cross-linked hydrogels can be produced through chemical agents [25,26]. This strategy is generally not compatible with entrapped proteins due to chemical modifications and loss of bioactivity. The collagen sponge used for rhBMP-2 delivery is chemically cross-linked in the absence of protein, and then adsorbs the rhBMP-2 solution in the operating room at the point of use [4,5]. Hydrogels can also be produced by physical cross-linking through repetitive peptide or polymer segments via hydrophobic/hydrophilic interactions [8]. This approach avoids the use of harsh chemical treatments, and can be more compatible with fragile protein therapeutics.

Silk fibroin is a natural protein that physically cross-links into hydrogel matrices [27,28]. Silk fibroin consists of disulfide bound heavy and light chains of ~370 kDa and ~25 kDa, respectively [29]. The primary structure consists of 12 hydrophobic protein domains rich in alanine and glycine residues with a high propensity towards forming anti-parallel β-sheet (physical cross-links) structure. These blocks are separated by 11 small hydrophilic amorphous domains. Silk fibroin possesses useful features as a biomaterial, including a history of biocompatibility, an all aqueous manufacturing process, controllable degradation rates and impressive mechanical properties [30–33]. Specifically, silk fibroin materials have demonstrated favorable immunological properties, a critical consideration when implanting protein-based materials [30,34–36]. Silk gel formation can be induced by several methods such as pH, temperature, shear, vortexing, electricity and sonication [27,28,33,37,38]. The use of silk-based biomaterials to stabilize and release proteins has been reported [39,40]. These studies revealed that proteins incorporated into silk films in trapped or untrapped forms. Untrapped protein interacted with silk in a reversible manner, releasing rapidly from the matrix, while in the trapped form irreversible interactions were found that required degradation of the silk matrix for release.

A new sustained delivery matrix, a lyogel, was produced by the lyophilization of antibody loaded silk hydrogels. The goal of the present work was to study the interactions between antibodies and lyogels to determine feasibility as a potential protein therapeutic delivery system. A murine monoclonal antibody was used as a pharmaceutically relevant model protein to assess entrapment and sustained release from silk lyogels. Sonication in the absence of antibody was utilized to induce hydrogel formation due to the method's potential for compatibility with fragile protein therapeutics [37]. The impact of lyophilization was evaluated by comparing antibody release rates between lyogels and the parent hydrogel material. Antibody release rates were substantially decreased in the lyogel compared to the hydrogel material above a threshold silk concentration. Finally, the material properties of the lyogels were investigated to gain insight into the mechanism of sustained release. In contrast to other hydrogel materials, silk fibroin is a highly hydrophobic molecule. Based on the hydrophobicity of silk and the observed swelling behavior of lyogels, it is hypothesized that rehydration resistance alters the nature of the reversible and irreversible antibody–silk interactions, which govern the rates of sustained release.

2. Materials and methods

2.1. Materials

Cocoons of *Bombyx mori* silkwork silk were purchased from Tajima Shoji Co., LTD (Sumiyashicho, Naka-Ku, Yokohama, Japan). Purified murine anti-TGFβ IgG1 monoclonal antibody was supplied by Genzyme Corporation (Framingham, MA). Clear Type I borosilicate glass serum vials for lyophilization were obtained from Wheaton Industries, Inc. (Millville, NJ). All chemicals were reagent grade purchased from Sigma–Aldrich (St. Louis, MO) or Mallinckrodt Baker, Inc. (Phillipsburg, NJ). All solutions were prepared using ultra pure water (UPW) with a 18.2 MΩ resistivity and <5 ppb TOC generated by a Millipore Milli-Q Advantage A10 purification system (Billerica, MA).

2.2. Lyophilized antibody powders

Antibody solutions at 5 mg mL^{-1} formulated in 0.02 M histidine buffer, 0.5% (w/v) sucrose, pH 6.0 were lyophilized in a LyoStar II tray freeze dryer (FTS Systems, Stone Ridge, NY). Each 5 mL serum vial was filled with 2.5 mL antibody solution and equipped with a vented silicone stopper. Samples were frozen to −45 °C and held for 8 h. Primary drying was performed at −20 °C, 100 mTorr for 40 h. Secondary drying was performed at 35 °C, 100 mTorr for 11 h. At the conclusion of lyophilization, the stoppers were depressed under a vacuum of 600,000 mTorr and the vials were sealed using aluminum tear off caps. Lyophilized antibody samples were stored at 5 °C ± 3 °C prior to use.

2.3. Concentrated silk fibroin solution preparation

Silk fibroin solutions were prepared using an aqueous process described previously [27]. Briefly, removal of the glue-like sericin protein was accomplished by boiling approximately 4 cm^2 silk cocoon pieces in a 0.02 M sodium carbonate solution for 60 min. After three ambient UPW rinses, the silk fibroin was air dried at ambient temperature for a minimum of 12 h. The dried fibroin was solubilized at 20% (w/v) in a 9 M aqueous LiBr solution at 60 °C for 60 min. This solution was dialyzed against UPW for 48 h using a 3500 MWCO Slide-A-Lyzer cassette (Thermo Fisher Scientific Inc., Rockford, IL). Silk concentrations were determined by comparing the mass of solution to the mass of dried silk after storage at 60 °C for 12 h. The silk concentration after dialysis was approximately 7.5% (w/w). Lower concentration silk solutions were prepared by dilution with UPW. Higher concentration silk solutions were prepared by dialysis against 20% (w/v) PEG (10,000 g mol^{-1}) at room temperature at a silk to PEG ratio of 1:33. Silk fibroin solutions were stored at 5 °C prior to use.

2.4. Preparation of silk hydrogels and lyogels

Silk hydrogels were prepared using the sonication method described previously [37]. Briefly, 8 mL of silk fibroin solution in a 15 mL conical tube was sonicated for 30 s using a Branson 450D sonifier equipped with a 3.175 mm diameter tapered microtip (Branson Ultrasonics Co., Danbury, CT). Sonication power was tailored for each silk concentration from 20% to 65% amplitude to achieve a sol–gel transition within 2 h. The sonicated solutions were cooled to room temperature by immersion in a room temperature water bath. Using a positive displacement pipette, 200 μL of the silk fibroin solution was transferred to a 96 well plate to make a single hydrogel pellet. The plates were allowed to sit at room temperature exposed to air until the solution turned opaque and water droplets formed on the surface, indicating the completion of the sol–gel transition. Lyogels were prepared by lyophilizing the hydrogel containing plates. Aluminum blocks were placed under the plastic plate to maximize heat transfer from the lyophilizer shelf. Lyophilization was carried out according to the parameters described above.

For antibody loaded constructs, lyophilized antibody was added to the cooled solutions to a target concentration of 5 mg mL^{-1}. The solution was gently inverted to ensure complete dissolution of the antibody powder and homogenous distribution. After thorough mixing, the solution was transferred to plates and allowed to gel as before. Based on this approach, the loading efficiency of antibody in hydrogels was considered 100%.

2.5. Swelling properties

Silk lyogels were immersed in phosphate buffered saline (PBS) pH 7.4 at 37 °C and sampled at defined time intervals. After removal of excess water by contact with a plastic surface, the weight of the rehydrated lyogel (W_r) was determined. The swelling ratio and fluid recovery were calculated as follows: