Published in final edited form as:

Macromol Biosci. 2013 March ; 13(3): 311–320. doi:10.1002/mabi.201200323.

Effect of Silk Protein Processing on Drug Delivery from Silk Films

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Abstract

Sericin removal from the core fibroin protein of silkworm silk is a critical first step in the use of silk for biomaterial-related applications, but degumming can affect silk biomaterial properties, including molecular weight, viscosity, diffusivity and degradation behavior. Increasing the degumming time (10, 30, 60 and 90 min) decreases the average molecular weight of silk protein in solution, silk solution viscosity, and silk film glass transition temperature, and increases the rate of degradation of silk film by protease. Model compounds spanning a range of physical-chemical properties generally showed an inverse relationship between degumming time and release rate through a varied degumming time silk coating. Degumming provides a useful control point to manipulate silk's material properties.

Keywords

biodegradable; biomaterials; controlled release; drug delivery systems; silk fibroin

Introduction

Controlled release polymer implants offer many potential advantages over traditional, periodic systemic injections including reduction or elimination of unwanted side-effects, enhanced efficacy and increased patient convenience and compliance.^[1] Biodegradable materials are particularly attractive for these applications as they require no surgical retrieval. However, biodegradable materials frequently lack the full scope of properties required for implantable drug delivery applications, including biocompatibility, non-immunogenicity, non-toxicity, high mechanical, thermal and chemical stability, tunable diffusivity to control release behavior, mild processing to preserve drug activity during encapsulation and stabilizing effects on the incorporated drug.^[2,3] Naturally-derived biodegradable materials generally offer superior biocompatibility and milder processing compared with synthetic degradable materials like PLGA,^[3] but their physical, chemical and mechanical properties are more difficult to tailor and they tend to release drug loads rapidly, known as burst release, over short time frames.^[2,4–5] Silk fibroin is a biologically-derived protein polymer isolated from domestic silkworm (*Bombyx mori*) cocoons that possesses unique properties to meet this need. Silk exhibits excellent biocompatibility^[3,7–9] and tunable, robust mechanical properties.^[6] Silk degrades to non-toxic products *in vivo* via proteolytic enzymes, and the degradation time course of silk implants can be controlled from days to years via regulation of beta sheet content (crystallinity) during processing.^[10–12] While many polymer drug carrier systems require harsh manufacturing conditions that can

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degrade or denature incorporated therapeutics (shear, heat, exposure to organic solvents or extreme pH), silk can be entirely aqueously processed using mild, ambient conditions.^[13–14] Silk has also been found to exert a stabilizing effect on encapsulated enzymes and antibodies, even at elevated temperatures.^[15–17] A wide range of silk material formats have been studied for controlled release applications.^[18–20]

B. mori cocoon silk is comprised of hydrophilic “glue-like” sericin proteins and the hydrophobic structural protein fibroin, which is comprised of a heavy chain (approximately 390 kDa) and a light chain (approximately 25 kDa).^[21–22] A key step in preparing many silk fibroin-based implantable biomaterials (including tissue engineering scaffolds and drug delivery devices) is degumming to remove the glue-like sericins. The degumming process typically relies on exposure to denaturing conditions (heat and/or pH) to accomplish the separation of sericin from the fibroin.^[23] Treatment of silk fibroin under alkaline conditions and heat encountered during degumming can also result in changes in the average molecular weight of silk fibroin protein fragments.^[13] The degumming time can potentially impact degradation rate as decreased molecular weight may disrupt ordered structures and reduce physical cross-links.^[13]

Relatively little research has been published on the effects of varied degumming conditions on silk protein and on the biomaterials formed from the regenerated silk fibroin protein. Pandit *et al.* found that degumming time (30, 60, 90, 120, 150 and 180 minutes) affected both average silk molecular weight and solution viscosity.^[24] Yamada *et al.* also reported decreased average molecular weight with increased degumming time,^[25] and Jiang *et al.* found that different degumming treatments produced clearly visible effects on the tensile behavior, mechanical properties and morphology of silk fibers.^[26] Rajkhowa *et al.* reported that an increase in intensity of silk degumming conditions (i.e. increased alkali concentration, treatment temperature or treatment time) reduced the average molecular weight of *Philosomiacynthia ricinieri* non-mulberry silk.^[27] A study of the effects of degumming on key silk material properties relevant to tissue engineering biomaterial applications was recently reported,^[23] where degumming time (5, 30 and 60 minutes) significantly affected the formation of 3D porous silk scaffolds, but did not affect macrophage activation or β -sheet content in the materials.^[23] The fact that β -sheet content was not affected by degumming time, while differences in degumming time dramatically impacted scaffold morphology further implicates the importance of protein molecular weight in silk material properties.

Silk materials exhibit drug release behavior dependent on diffusion of the drug through the polymer matrix, the degradation of the matrix, or a combination of both mechanisms.^[28] Control of both diffusion through and degradation of the silk matrix by manipulation of the silk properties and processing have been demonstrated. Drug diffusion from silk drug carriers has been controlled via carrier morphology (including geometry, coating thickness, porosity)^[29] and manipulation of the crystallinity/beta-sheet content.^[30–31] Degradation rate of silk is dependent on many factors, including material properties (organic solvent vs. aqueous processing, crystalline content, silk concentration and porosity),^[10–11] silk material format,^[32–33] type and concentration of proteolytic enzyme^[32,34] and immune system response.^[11]

Despite the potential for varied degumming conditions to impact properties of silk that are key to its use in controlled release drug delivery applications, the effect of this critical processing step on drug release and carrier degradation has not been addressed in detail. Previous studies have consistently shown that increased degumming time resulted in decreased average molecular weight of the silk protein,^[23–25] and for many biodegradable polymers, molecular weight is a key factor in controlling degradation and drug release

kinetics.^[35–36] Polymer molecular weight relates to chain length, which can impact the rate of diffusion through polymer matrices and rate of polymer degradation.^[36] Varying the molecular weight of a polymer can therefore be used to control both degradation rate and the release kinetics of drug from the polymer.^[36]

The impact of molecular weight on drug release and degradation has been demonstrated for several polymer delivery systems including poly (lactic-co-glycolic) acid (PLGA),^[35] poly (D,L-lactic acid) (PDLA),^[37] *N*-isopropylacrylamide (NIPAAm),^[38] polycaprolactone (PCL)^[39] and silk fibroin.^[40] Fang *et al.* examined the release of buprenorphine from bulk loaded 12% (w/v) silk hydrogels and found that hydrogels prepared from a 5:1 blend of higher molecular weight silk (average MW = 76 kDa) to lower molecular weight silk (average MW = 18 kDa) (the method for controlling molecular weight was not reported) released approximately 59% of the total drug after 4 days, compared with 26% total drug released after 4 days from hydrogels prepared entirely from the high molecular weight silk.^[40] Replacing only one-sixth of the silk in the 12% (w/v) hydrogel with a lower molecular weight silk resulted in more than doubling the release rate of the small molecule drug, which suggests that the molecular weight of silk could play a key role in regulating diffusion-driven release. If varied degumming impacts drug diffusion through and/or degradation of the silk, it could provide a controllable parameter through which tunable implant behavior could be dialed in via manipulation of processing. However, there are also cases in the literature where the rate of drug release and the rate of polymer matrix erosion are independent of initial molecular weight.^[41] For example, the rate of release of carmustine (BCNU) from GLIADEL® wafers (copolymer matrix consisting of 1,3-bis (p-carboxyphenoxy) propane (CPP) and sebacic acid (SA)) and the rate of wafer erosion were both independent of the initial molecular weight.^[41]

The objective of the present study was to determine the effect of degumming time and on silk material properties relevant to controlled drug delivery applications, including average molecular weight, viscosity, packing density, diffusivity and degradation.

Experimental Section

Silk Fibroin Solutions

Cocoons of *Bombyxmori* silkworm silk were purchased from Tajima Shoji Co., LTD (Sumiyoshicho, Naka-ku, Yokohama, Japan). Silk fibroin was prepared from *B. mori* cocoons as we have previously described.^[42] Briefly, cocoons were degummed for 10, 30, 60 or 90 minutes by boiling in a solution of 0.02 M Na₂CO₃. Degummed fibroin was rinsed and then dried at ambient conditions overnight. The dried fibroin was solubilized in a 9.3 M aqueous LiBr, dialyzed against distilled water for 2.5 days using Slide-a-Lyzer dialysis cassettes (MWCO 3,500, Pierce Thermo Scientific Inc., Rockford, IL) and centrifuged. Silk fibroin concentration (in w/v) was determined by evaporating water from a solution sample of known volume. All solutions were stored at 4–7°C before use.

SDS-PAGE Gel Electrophoresis

Samples of silk prepared using varied degumming times (10, 30, 60 or 90 minutes) were analyzed by SDS-PAGE using NuPAGE Novex 3–8% Tris-Acetate pre-cast gels and Tris-acetate running buffer. Five micrograms of protein per well were run as denaturing, reduced samples according to the manufacturer's instructions (150 V constant for 1 hour). Separated proteins were detected with Colloidal Blue staining according to the manufacturer's instructions. Molecular weight was estimated from HiMark unstained protein standard. All SDS-PAGE gel materials were purchased from Invitrogen (Carlsbad, CA).

Viscosity

Kinematic viscosities (in centistokes, or cSt) of 7% (w/v) silk solutions were measured at room temperature using Ubbelohde viscometers according to the manufacturer's instructions. Each solution was measured in triplicate (n=3). The solutions prepared from silk cocoons exposed to 30, 60, and 90 min degumming times were measured using a 4–60cSt range viscometer (SI Analytics GmbH, Woburn, MA). Due to its higher viscosity, the solution prepared from silk cocoons exposed to a 10 min degumming time was measured using a 60–300cSt range viscometer (Cannon Instrument Company, State College, PA).

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) studies were carried out using TA Instruments Q100 DSC (New Castle, DE), and equipped with a refrigerated cooling system. Indium was employed for the temperature and heat flow calibration. The heat capacity was evaluated with respect to sapphire standard. Dry nitrogen gas was purged into the DSC cell with a flow rate of 50 mL/min. Five mg of each silk sample were encapsulated in aluminum pans and first heated to 140°C to remove the bound water in the materials,^[43–45] and then standard mode DSC measurements were performed at a heating rate of 2°C·min⁻¹, from –40°C to 350°C. Endotherms are presented with downward deflection in scans.

Packing Density

Packing density was determined by measuring mass using an analytical balance, and measuring film thickness using a Mitutoyo absolute digimatic caliper with a 0.01 mm resolution (Mitutoyo America Corporation, Aurora, IL). Density (in mg·mm⁻³) was determined by dividing the total film mass (in mg) by the total film volume (mold surface area in mm² multiplied by film thickness in mm). For the packing density studies, n = 5. Significance was characterized with a two-tailed t-test (df = 8).

Mass Loss

Protease type XIV (3.5 units·mg⁻¹) from *Streptomyces griseus* was purchased from Sigma-Aldrich (St. Louis, MO). Films were prepared from 6% (w/v) silk that had been degummed for various times (10, 30, 60 or 90 min). Films were prepared by aliquoting silk solution into Teflon coated molds, drying overnight at ambient conditions, and then treating the dried films with methanol for 5 minutes to induce physical (beta sheet) cross-linking. The film masses were determined using an analytical balance. To evaluate mass loss, films were immersed in 1 mL of phosphate buffered saline (PBS) or 1 mL of 0.1 mg·mL⁻¹ protease type XIV solution for 5 days. Every 24 hours, a subset (n=3) of the films was removed, gently rinsed, dried overnight at ambient conditions and reweighed. Protease solution and PBS were replaced every 24 hours to account for proteinase activity loss due to 37°C incubation.^[10] The fraction of remaining mass was determined by dividing the mass at each time point by the initial mass values.

Model Compound Release

Silk Film Reservoir Preparation and Capping—To characterize the effects of the varied degumming time on release kinetics, model compounds were chosen to represent a range of molecular weights, aqueous solubilities and potential therapeutic classes (including both small molecules and proteins): azoalbumin, reactive-red 120, rifampicin, and indigo carmine, which are 66.4 kilodaltons, 1469.98 g·mol, 822.94 g·mol and 466.35 g·mol, respectively. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Compound loaded silk fibroin films were prepared as we have previously described.^[30] Briefly, compound solution was mixed with silk solution (degumming time of 60 minutes)

to a final concentration of 6% (w/v) silk and aliquoted into 24 well plate wells (0.5 mL per well). Controls were prepared using silk diluted with distilled water. Films were dried overnight at ambient conditions then treated with methanol for 5 minutes to increase β sheet content and render the films water insoluble.

Due to its limited solubility in water, bulk loaded rifampicin film reservoirs were prepared using an alternative protocol: 0.25 mL of 8% (w/v) silk was aliquoted into each well of a 24 well plate, then 0.25 mL of 10 mg/mL rifampicin in methanol was added and mixed into each well (resulting in 0.5 mL of 4% (w/v) silk hydrogel containing 2.5 mg of rifampicin per hydrogel). The addition of methanol to silk induced gelation, and the resulting gel was dehydrated to a film overnight at ambient conditions. The dried films were not subjected to any additional methanol treatment, as the necessary cross-linking occurred during gelation.

Once the aqueous or hydrogel-derived film reservoirs were treated and dried, 0.5 mL of 6% silk that had been degummed for varied times (10, 30, 60, 90 min) was added to each compound-loaded film, forming a capping layer. Capping layers were dried overnight at ambient conditions, then treated with methanol for 5 minutes. For comparison, samples of each compound-loaded film were also left uncapped. Azoalbumin, rifampicin and reactive-red 120 loaded films all received only one capping layer. Due to its relatively low molecular weight, indigo carmine films received either two or four capping layers (abbreviated 2 $\times$ or 4 $\times$). For multiple capping layers, each layer was dried and treated with methanol prior to the addition of the next layer.

Release Profiles—To determine compound release from the capped silk films, 1 mL of PBS was added to each well and samples were incubated at 37°C. At desired time points, the buffer was removed and replaced with fresh buffer. The amount of released compound was determined using UV-Vis spectroscopy at 358 nm, 475 nm, 535 nm and 610 nm, for azoalbumin, rifampicin, reactive red 120 and indigo carmine, respectively. The amount of released compound in each sample was summed with the amounts at each previous time point and divided by the total amount to obtain cumulative release values. Four samples were tested (n=4) and each sample was assayed in triplicate.

Results and Discussion

Effect of Degumming Time on Silk Fibroin Molecular Weight

Figure 1 shows the results of SDS PAGE analysis of silk degummed for 10, 30, 60 and 90 minutes. For SDS-PAGE of untreated controls (non-degummed silk cocoon fibroin and silk from the silkworm gland) see [25]. Increased degumming time resulted in a decrease in the silk fibroin average molecular weight, as evidenced by migration of the smear further down the gel with increasing degumming duration. Silk degummed for 10 minutes had a molecular weight distribution predominantly in the 171 kDa to 460 kDa range, 30 minute degumming produced silk with a broad molecular weight distribution from approximately 31 kDa to 268 kDa (the lightness of the protein smear in the 30 minute lane is due to the even distribution of the protein over a larger range than the 90 minute, 60 minute or 10 minute treatment groups, as protein loading was consistent across lanes). The majority of the silk protein produced by the 60 minute degumming time was less than 171 kDa and the 90 minute degumming time produced silk proteins that were predominantly 71 kDa or smaller. These data confirm previous reports that treating silk fibroin under the heated alkaline conditions encountered during degumming impacts the molecular weight.^[13, 23–25]

Effect of Degumming Time on Aqueous Silk Solution Viscosity

Viscosity values for aqueous silk solutions prepared from silk cocoons exposed to varied degumming times are shown in Figure 2. Silk solution viscosity increased with decreased degumming times, consistent with previous reports.^[24] For degumming times from 30 minutes to 90 minutes, the proportional relationship observed between degumming time and viscosity (Figure 2B) is consistent with the Mark-Houwink equation which predicts that viscosity (η) will increase proportionally with weight-average molecular weight (M_w).^[47] The viscosity of the 10 minute degumming time silk solution is considerably higher than would be expected if the proportional relationship observed above a 30 minute degumming time were upheld for shorter degumming durations, suggesting the relationship between degumming time and viscosity is non-linear. An alternate explanation is that the higher molecular weight fragments in solution prepared from silk degummed for 10 minutes began to transition from solution state to a partially crosslinked state, even when stored at 4°C, as decreased degumming time reduced gelation times (data not shown).

Effect of Degumming Time on Silk Film Glass Transition Temperature

DSC analysis and glass transition times for silk films cast from silk solutions prepared using varied degumming times are shown in Figure 3. Glass transition temperature decreases linearly relative to increasing degumming time (i.e., as average silk fragment size decreases, the glass transition temperature also decreases). This is consistent with literature on PLGA, where T_g increases with increasing molecular weight due to the presence of fewer chain ends in the higher molecular weight polymer.^[47–48]

Effect of Degumming Time on Silk Film Packing Density

Films prepared from silk degummed for 10, 30, 60 and 90 minutes had packing densities of 0.92 ± 0.05 , 0.92 ± 0.03 , 0.95 ± 0.05 and 0.94 ± 0.02 mg/mm³, respectively. Statistical analysis revealed no significant difference in packing density among the different degumming times (two tailed t-test, $df = 8$, $p < 0.01$). This suggests that, despite the differences in average molecular weight and solution viscosity, degumming time does not affect initial packing density or film thickness. For the range of degumming times tested (10–90 minutes), films cast from silk solutions of equal protein concentrations produce equivalent path lengths for drug diffusion regardless of degumming duration.

Effect of Degumming Time on Silk Film Proteolytic Degradation

Mass loss of films prepared from varied degumming times was studied in PBS and protease type XIV solutions to determine the effect of degumming time on rate of silk carrier degradation (Figure 4). As expected, little mass loss occurred for films in PBS (approx. 5–20%) due to the absence of proteinase (mass loss that occurs in PBS is the result of uncrosslinked silk dissolving/diffusing out of the film, as carrier degradation will not take place in the absence of proteinase) (Figure 4A). In protease type XIV solution, a direct relationship was observed between degumming time and degradation rate: the longer the degumming (and the smaller the average silk fragment size), the more rapid the proteolytic degradation (Figure 4B). After 5 days in protease solution, films cast from silk degummed for 90 minutes have almost entirely degraded, while approx. 20%, 50% and 70% of the films' average initial mass still remains for films cast from 60 minute, 30 minute and 10 minute degummed silk, respectively.

Effect of Degumming Time on Indigo Carmine Release through Silk Capping Layers

Figure 5 shows the cumulative release behavior for films loaded with indigo carmine, then capped with either two or four capping layers of silk prepared using varied degumming times. When indigo carmine loaded films were coated with two silk capping layers, the

difference in release profile among the different degumming times was minimal. However, when the number of capping layers was increased to four (Figure 5B), a difference in release behavior becomes more pronounced. For films receiving four silk capping layers, release rate decreased with decreased degumming time.

Though further study is needed before the system can be accurately modeled and controlled, the release data demonstrate qualitative increases in the rate of compound release through silk as the degumming time increased and identify key candidate characteristics to investigate and improve further, such as capping thickness. .

Effect of Degumming Time on Rifampicin, Reactive Red 120 and Azoalbumin Release through Silk Capping Layers

Figure 6 shows cumulative release behavior of silk films loaded with rifampicin, reactive red 120 and azoalbumin then capped with a single layer of silk prepared using varied degumming times. As with indigo carmine release through four capping silk layers, the release rate of rifampicin through one silk capping film increased with increased degumming time, suggesting the barrier to diffusion increased with decreased degumming time. The difference in rate of compound diffusion through the silk is apparent for rifampicin compared to indigo carmine even though the total capping thickness was thinner, likely due to the difference in compound properties, particularly molecular weight and aqueous solubility. In the case of reactive red 120, increased degumming times again result in an increased rate of compound diffusion through the capping layer, with a pronounced difference in release behavior resulting when degumming time increased from 60 minutes to 90 minutes. The reason for this effect requires further investigation, but may relate to a critical lower threshold for silk molecular weight. Increased degumming times only result in an increase in the rate of azoalbumin diffusion through the capping layer up to 60 minute degumming times: no observable difference exists between the release curves for the films capped with 60 and 90 minute degumming time silk films. The difference in azoalbumin release behavior for films capped with silk degummed for 60 minutes and 90 minutes was not statistically significant, but was statistically significant for all other groups (two tailed t-test, $df = 4$, $p < 0.05$). .

Discussion

Results of the SDS-PAGE analysis demonstrated that increased degumming time decreased the average molecular weight of silk fibroin. This result is consistent with previous reports.^[23–25] During degumming, the sericin protein is hydrolyzed and the amide bonds are broken, resulting in smaller fractions which are dispersed and solubilized in the hot alkaline degumming solution, enabling separation of the glue-like sericin from the fibroin protein. Extending the exposure of the silk to heat and alkalinity also induces hydrolytic degradation of the water-insoluble silk fibers, breaking the silk fibroin protein chains into smaller fragments.^[49–50]

Data presented here suggest that degumming process must be taken into account during protein preparation for use in silk biomaterials for drug delivery, as it can impact the average molecular weight of the purified fibroin, which may in turn affect the material properties.

Given that silk is a natural product, some amount of batch to batch variability is anticipated, which further highlights the need to characterize the effects of processing and control the degumming process. While distribution of protein fragments on an SDS-PAGE gel does not represent a quantitative method for standardization, Wray et al. describe a fluorescence spectroscopy method that can be used to reproducibly distinguish between silk samples with different molecular weights.^[23] Also, because a linear relationship is observed for

degumming times greater than or equal to 30 minutes, solution viscosity could be used as an indirect measure of molecular weight, and might therefore represent a more quantitative parameter than the smears on SDS-PAGE.

Increased degumming time used in preparing the silk decreased the diffusivity of the silk film to the model compound tested, increased their release rates from capped silk films. Differences in the release behavior of the compounds tested (indigo carmine, rifampicin, azoalbumin and reactive-red 120) were influenced by compound properties, including molecular weight, aqueous solubility and affinity for the silk carrier. The model compounds (indigo carmine, rifampicin, reactive red 120 and azoalbumin) exhibit incomplete release from the capped film reservoirs (i.e. cumulative release below 100% total loading), an effect which is attributed to interaction of silk protein with the encapsulated compound, and which is anticipated to vary with compound affinity for silk. Unbound compound or protein exhibits diffusion-driven release from the silk matrix, while release of the bound fraction will be dependent on proteolytic degradation of the silk carrier. The total proportion of freely diffusible compound appears to vary with degumming time, with the proportion of total recovery generally increasing as degumming duration increases. This suggests that degumming also impacts the affinity of compound for the silk and, by extension, the total fraction available for diffusion from the carrier and the total fraction bound that will rely on proteolysis-driven release. Further work is needed to assess compound binding to silk and the effect of degumming time on compound-silk interactions, as these can potentially significantly impact release kinetics.

Longer degumming durations degraded the silk protein into increasing smaller fragments, as shown by SDS-PAGE, and silk films cast from silk prepared using longer degumming times became increasingly permeable to drugs. FTIR analysis has shown (both qualitatively and quantitatively) that degumming time has no effect on β sheet content.^[23] A potential explanation for this observation, represented schematically in Figure 7, is that the hydrophobic domains of silk involved in β -sheet secondary structure are unaffected by increased degumming time, while the other regions of the silk protein are increasingly degraded during degumming (see [23] for a more detailed schematic of the degumming-induced degradation of the silk protein structure). When films are cast from silk solution prepared with varied degumming times, β -sheet content remains constant but in films prepared from longer degumming time silks are less organized and more permeable. The decreased organization and increased permeability associated with increased degumming time might also explain the increase in rate of mass loss in proteinase buffer, as the steric hindrance to enzymatic degradation is also likely decreased.

Conclusion

Degumming time impacts silk material properties (including average fragment size, solution viscosity and glass transition temperature). The degumming process can also affect the rate of compound diffusion through silk films and the rate of silk degradation, critical properties to characterize and manipulate for drug delivery applications. Degumming time therefore represents a new, previously unexploited control point for manipulating silk drug carrier material properties with potential utility in studying and designing silk biomaterials for controlled release. The observation that varied degumming conditions produce significant changes in release behavior and degradation without impacting the β sheet content is particularly relevant, as it suggests that additional control of release rate can be achieved by manipulating both the β -sheet content and molecular weight of the silk matrix.

Acknowledgments

This project was supported by grant R01NS058780 from the National Institute of Neurological Disorders and Stroke and by the Epilepsy Research Foundation through the generous support of the Arlene and Arnold Goldstein Family Foundation. The authors would like to thank Lindsay Wray for advice and assistance with SDS-PAGE gels and Anthony Barry for discussions and technical guidance.

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Figure 1.

SDS-PAGE analysis of silk subjected to various degumming times and stained with colloidal blue. Lane 1: HiMark high molecular weight protein standard; lane 2: empty; lane 3: silk degummed for 10 minutes; lane 4: silk degummed for 30 minutes; lane 5: silk degummed for 60 minutes; lane 6: silk degummed for 90 minutes.

Figure 2.

Kinematic viscosity (in centistokes) versus degumming time (A) Kinematic viscosity (in centistokes) for degumming times of 10, 30, 60 and 90 min (B) Kinematic viscosity (in centistokes) for degumming times of 30, 60 and 90 min. N=3, error bars represent standard deviation. Where error bars are not shown, the standard deviation falls into the background.

Figure 3. Effect of degumming time on the glass transition temperature of silk films: (A) DSC analysis of silk films prepared from silk degummed for 10, 30, 60 and 90 minutes. (B) Glass transition temperature versus degumming time.

Figure 4.

Mass loss over 5 days of silk films cast from silk prepared using varied degumming times immersed in (A) PBS or (B) 0.1 mg/mL protease type XIV solution. N=3, error bars represent standard deviation. Where error bars are not shown, the standard deviation falls into the background.

Figure 5. Cumulative indigo carmine release from films (A) capped with two silk layers and (B) capped with four silk layers with silk prepared using varied degumming times. N=4, error bars represent standard deviations (where error bars are not shown they fall into background).

Figure 6.

Cumulative release of (A) rifampicin (B) reactive red 120 and (C) azaolabumin from silk gel films capped with a single layer of silk prepared using varied degumming times. N=4, error bars represent standard deviations (where error bars aren't shown they fall into background).

Figure 7.

Schematic of the relationship between β -sheet content, fragment size and diffusivity of silk films. At the beginning, all three aqueous silk solutions produced by the different degumming times have the same total amount of red bars (representing the domains involved in β sheet formation) but with increasing degumming time the other regions of the silk protein (e.g., the amorphous linkers, represented by gold bars) are increasingly degraded. When silk films are formed around the drug reservoir (silk coating in orange, drug reservoir in green), all the films have the same β sheet content. Increasing degumming time produces decreasing molecular weight fragments, which results in the formation of a less organized, more permeable silk membranes for compound diffusion.