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Release of Bovine Serum Albumin from a Hydrogel-Cored Biodegradable Polymer Fiber

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Abstract: *We have developed a novel biodegradable, polymeric fiber construct that is coextruded using a wet-spinning process into a core-sheath format with a polysaccharide pre-hydrogel solution as the core fluid and poly(L-lactic acid) (PLLA) as the sheath. The biodegradable, biocompatible fibers were extruded from polymeric emulsions comprised of solutions of various molecular weights of PLLA dissolved in chloroform and containing dispersed, protein-free aqueous phases comprising up to 10% of the emulsion volume. Biologically sensitive agents can be loaded via a dispersed aqueous phase in the polymer, and/or directly into the polysaccharide. We show that this core-sheath fiber format will load a model protein that can be delivered for extended periods in vitro. Bovine serum albumin (BSA) was loaded into the fiber core as a model protein. We have shown that the greater the volume of the protein-free aqueous phase dispersed into the polymeric continuous-phase emulsion, the greater the total release of BSA encapsulated by a core gel comprised of 1% sodium alginate solution. We conclude this fiber format provides a promising vehicle for in vivo delivery of biological molecules. Its biocompatibility and biodegradability also allow for its use as a possible substrate for tissue engineering applications.* © 2006 Wiley Periodicals, Inc. Biopolymers 81: 419–427, 2006

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INTRODUCTION

Several strategies have been used to successfully modify and bridge a central nervous system (CNS) lesion to enhance axonal regeneration. These include transplantation of fetal CNS tissue, Schwann cells, olfactory ensheathing cells, neurotrophin secreting cell-lines, and peripheral nerves.[1–3] Although these graft types typically provide axonal regeneration of a few hundred microns, functional recovery is nearly non-existent. For example, autografts provide the best regeneration results and are the clinical standard, but they pose the disadvantage of the sacrifice of a healthy nerve, leading to permanent denervation of the donor site.[3] Additionally, whenever the central nervous system is damaged, the injury response that occurs results in the formation of a glial scar through a process called reactive gliosis.[4] Disadvantages like this have led to the experimental development of artificial nerve grafts.[5–7]

Artificial grafts comprised of bioresorbable microfilaments provide good biocompatibility and biodegradability.[8] Such grafts also provide initial structural support for regenerating axons, while degrading over time to provide space for growth. In theory, microfilaments may also aid the restructuring of the nerve by aligning axon growth, and may eliminate the morbidity associated with autogenous nerve grafting.[9] When used as drug carriers, microfilament grafts can also provide a way to modulate cell behavior in proximity to the fibers as the drug diffuses from the fiber to cells that are relatively close to the fiber.

It has been one of the main goals in our laboratory to develop suitable biodegradable substrate materials upon and/or within which nerve cells can be supported for directional growth. For example, we have created fibers for use as tissue growth substrates using a patented wet extrusion technique in which the polymer solution incorporates an aqueous emulsion.[10–12] The key point of using an emulsion in our process is that sensitive biological molecules, such as proteins, growth factors, cytokines, enzymes, etc., carried by the emulsion are only exposed to an aqueous, biologically friendly environment during extrusion. They are protected almost completely from the frequently harsh chemical or temperature environments of standard extrusion processes.

Expanding upon our emulsion-loaded fiber concept, we have developed a technique for extruding a bicomponent polymer fiber containing a gel or hydrogel within its core. As a tissue substrate, this fiber construct allows for cell growth either within its hydrogel-containing core, on the outer surface, or through a combination of the two. As a drug delivery device, this fiber construct vastly increases the aqueous phase loading capability of our emulsion-loaded fibers, as well as the quantity and diversity of sensitive biological molecules that can be loaded into a given fiber length. We have co-extruded xanthan-galactomannan and sodium alginate gels in such fibers, with alginate preferred as a core material for this particular application.

Alginate is a natural polysaccharide gel derived from brown algae or kelp. It is composed of (1-4)-linked β-D-mannuronic acid and α-L-guluronic acid monomers arranged in block structures. Alginate has many applications in the food and pharmaceutical industries, and in medical research it has been used as a material for the sustained release of bovine serum albumin (BSA), fibroblast growth factor (FGF), immunoglobulin G (IgG), nerve growth factor (NGF), transforming growth factor α (TGF-α), TGF-β, live cells, and more.[13–15] Alginate also has been used as scaffold material for tissue engineering applications,[16] has been used to encapsulate Schwann cells,[17] and most importantly to our research, has been shown to enhance elongation of amputated axons of infant rat spinal cords.[18–20]

We have created a biodegradable fiber format capable of encapsulating a variety of biologically sensitive molecules and/or carrying drug-loaded microspheres or lipospheres. We loaded BSA into our alginate solutions prior to fiber extrusion, and then examined the release of this protein into supernatant over a period of approximately 80 days. Our investigations have shown promise for the use of this fiber construct as a drug delivery device and tissue substrate.

MATERIALS AND METHODS

The development of a hydrogel-cored PLLA fiber required the use of standard polymeric fiber wet extrusion methods, along with internal gelation techniques for in situ formation of the alginate gel core of this fiber. The core of the fiber was loaded with a model drug (BSA) prior to extrusion in order to assay its release into prepared supernatant over a chosen period of study. A patent pending technique was followed for the formation of these fibers.[21]

Materials

Three molecular weight PLLA pellets, 2, 100, and 300 kDa, were obtained from Polysciences (Warrington, PA). All other chemicals were purchased from standard laboratory suppliers unless otherwise noted and were American Chemical Society (ACS) or high performance liquid chromatog-

raphy (HPLC) grade. All solutions used for in vitro assays were of tissue culture grade.

Alginate Stock Preparation

Sodium alginate stock solution for all fiber extrusions was prepared in advance of all other working solutions by slowly adding 5.0 g of Na-alginate powder (Spectrum Chemical, Gardena, CA) into the vortex created by stirring 500 mL of reverse osmosis/deionized (RO/DI) water in a glass beaker at medium speed using a stir plate and a magnetic stir bar. This solution was then covered and allowed to dissolve under continuous stirring overnight. Once completely dissolved, the resulting solution, which was prepared at 1.0% w/v, was filtered through a series of five hydrophilic cellulose nitrate membranes to remove suspended particles and clarify the solution. The filtration was accomplished in stages, first through a 3-μm membrane, followed by a series of smaller pore membranes, and ending with a final filtration step in an aseptic environment through a 0.2-μ syringe filter. The filtration process yielded a clarified and filter-sterilized alginate solution, which was then aliquoted into sterile 50-mL Falcon tubes and stored at 4°C for later use.

Release Media Preparation

A solution of 0.05*M* Tris buffer at pH 7.4, supplemented with 2.5 m*M* $CaCl_2$, was prepared and heat sterilized. Once completely cooled, 1% penicillin/streptomycin was added, and the solution was then sterile filtered into 50-mL Falcon tubes. These were frozen at −20°C for use in release studies. When needed, a fresh tube of release solution was thawed at room temperature and used to replace assay supernatant as each time point. This ensured that fresh penicillin was available at each media replacement.

Polymer Preparation

The polymer solution for this study was a blend prepared from 100, 2, and 300 kDa pellets of poly(L-lactic acid). We prepared a starting solution by dissolving an appropriate amount of the noted polymers in chloroform to yield a 15% w/v solution that was 80% by weight 100 kDa PLLA, 15% by weight 2 kDa PLLA, and 5% by weight 300 kDa PLLA. The polymer pellets were placed into a snap-top glass vial, 5 mL of chloroform was added, the vial was sealed with Parafilm®, and the solution was stirred at medium speed for 4 h. Once completely dissolved, the polymer solution was stored overnight at 4°C.

The following morning, iso-octane was slowly added to the prepared polymer solution to yield a 65/35 chloroform/iso-octane volumetric ratio in the final product. Iso-octane is a nonsolvent for PLLA, and its addition brings the final polymer solution close to its cloud point. Addition of the iso-octane was accomplished in volumetric thirds, with vortexing between additions to ensure proper blending of the solutions. In this particular case, a total volume of 2.692 mL of iso-octane was added to the chloroform and polymer solution. This complete polymer plus iso-octane solution was stirred for an additional 30 min to ensure blending of the iso-octane into the solution.

Following stirring of the polymer solution, 1% w/v of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC; Avanti Polar Lipids, Inc., Alabaster, AL) was added as a surfactant, and allowed to dissolve completely. The solution was stirred for an additional 10 min to ensure solubilization of the DPPC. The polymer solution created was used for all fiber extrusions accomplished for this study.

BSA Loading and Alginate Gelation

To prepare fiber treatments containing loaded protein, 0.06 g of BSA was added to 12 mL of 1% sterile-filtered sodium alginate solution and allowed to dissolve for 6 h. Once dissolved, the solution was sterile filtered in an aseptic environment through a low protein binding, 0.2-μm syringe filter into a sterile 15-mL Falcon tube and stored for use within fibers containing BSA-loaded alginate cores. Stock, sterile-filtered, 1% alginate solution was similarly stored for use within blank control fibers.

Immediately before extrusion of the gel-loaded fibers, 3 mL of the appropriate alginate solution was loaded into a sterile syringe (BSA containing or blank). In an aseptic environment, 0.0045 g of ultraviolet (UV)-sterilized $CaCO_3$ was then added to the alginate, yielding a 15 m*M* final concentration.[22,23] The syringe was then appropriately sealed, and the assembly was vortexed at medium speed for 30 s to homogeneously distribute the calcium carbonate. $CaCO_3$ is virtually nonsoluble in water at neutral pH, and because calcium ions are not freed when it is added to the alginate solution, gelation is not initiated.[24]

For a 30 m*M* glucono-δ-lactone (GDL) concentration, 0.0160 g was added to the alginate/$CaCO_3$ solution just before the actual fiber extrusion. GDL is readily soluble and slowly hydrolyzes upon its addition to the alginate solution, which results in slow but continuous lowering of pH. This change in pH dissociates Ca^{2+} from $CaCO_3$, which forms H_2O and CO_2 upon the addition of the two protons. The released Ca^{2+} initiates gelation of the alginate. The 2:1 molar ratio of the GDL to $CaCO_3$ ensures the final gel has a neutral pH and protects the drug from potentially damaging acidic environments. Their absolute molar concentrations affect the final properties of the alginate gel and its rate of gelation.[25] Weak gelation occurs in approximately 30 min under the conditions used in this study.

Emulsion Loading

Polymeric emulsions were created before fiber extrusion by dispersing sterile DI H_2O into an appropriate volume of prepared polymer solution containing 1% DPPC as a surfactant. The emulsification technique followed similar methods used to create emulsion-loaded microspheres.[26,27] The wet extruded fibers used in this study contained either 0, 5, or 10% emulsions by volume within their polymer sol-

utions. Emulsions were created by probe sonicating these volumes into the respective polymer solution at 50 W (20% amplitude) for a total of 40 s using a 5–0.5-s on–off duty cycle. An ice water bath was used to limit the heating of the mixture during sonication to avoid solvent evaporation and other temperature affects on the polymer. We, and others, have examined the effects of probe sonication on protein and polymer stability, and we have concluded this emulsification technique is minimally detrimental to the fiber and its incorporated protein.[12,28,29]

The 5% v/v emulsion fiber used in this study was prepared by adding 121 μL of RO/DI H_2O into 2.3 mL of polymer solution in a single addition. The 10% v/v emulsion fiber used in this study was prepared separately and only prior to its extrusion by adding 255 μL of RO/DI H_2O into 2.3 mL of polymer solution in a single addition. Respectively, these mixtures were then pulse sonicated as noted, with the probe tip cleaned between sonications, and then drawn into 5-mL gas-tight syringes (Hamilton Co., Reno, NV) using an 18-gauge needle. The emulsions were then allowed to degas within the syringes at room temperature for 15 min prior to extrusion. This step also verified the stability of the respective emulsion and allowed the polymer solution to relax and further stabilize prior to extrusion. The 0% emulsion was similarly prepared, sans aqueous phase.

Fiber Extrusion

In order to concurrently extrude the respective polymer and alginate solutions (with and without BSA), the loaded syringes containing these solutions were placed into linear syringe pumps (KD Scientific Model 200), and appropriate fittings installed onto each in order to connect the syringes into a dual-lumen spinneret designed for these extrusions. The spinneret assembly consisted of a nylon T-fitting onto which a blunt-tipped, 18-gauge, stainless steel needle and a male luer fitting containing a rubber septum were connected. The assembly's remaining fitting accepted a male luer connection. A 12-in. section of 24-gauge hypodermic tubing pierced the septum and was maintained concentric within the bore of the needle by a loose coil of thin wire of the diameter of the annular space. The syringe containing the alginate solution was connected to this hypodermic tubing via a luer lock fitting containing an integral silicon septum. The syringe containing the polymer solution was connected to the spinneret assembly using a section of sterile Viton® tubing capped with male and female luer lock fittings. Figure 1 provides a graphic depiction of the extrusion apparatus.

Extrusion of all fibers was accomplished by immersing the tip of the spinneret assembly through a septum sealing a coagulating bath of pentane contained within a 250-mL Erlenmeyer flask. The linear syringe pumps were started, allowing the alginate solution to flow through the hypodermic tubing and out the tip of the spinneret, and the polymer solution to flow into the T-fitting, around the hypodermic tubing, and out the tip of the spinneret. As the polymer and alginate solutions were extruded concurrently out of the

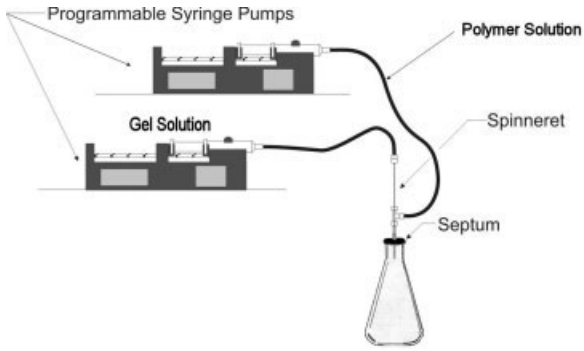

FIGURE 1 Diagram of the extrusion apparatus used to create the fibers discussed in this article. Polymer and solutions contained in syringes were concurrently extruded using two linear syringe pumps. Appropriate fittings and tubes connected each syringe to a dual-lumen spinneret designed for these extrusions.

spinneret, the polymer contacted the coagulant, resulting in the diffusion of chloroform out of and pentane into the polymer solution. Pentane is not miscible with water, so both the dispersed aqueous phase in the emulsion loaded fibers, as well as the alginate solution, remain almost completely free from solvent penetration. Pentane is a nonsolvent for PLLA, yet very miscible with chloroform. Therefore, as the chloroform leaves to enter the pentane, it increases the polymer concentration. At the same time, pentane enters the polymer stream and begins to cause the polymer to precipitate from solution. Both events have the net result of forming a congealed polymer sheath. As this sheath moves through the coagulant, it continues to harden, and fully encapsulates and supports the gel core. Extruded fiber was allowed to collect in the bottom of the flask until the respective extrusion was complete. At that time, the flask containing the resulting fiber and its pentane coagulant was tightly capped with a rubber stopper and stored at 4°C overnight. Continued exposure to pentane during storage allowed the extraction of residual chloroform from the fiber, while gelation of the alginate core continued through the action of the loaded GDL and $CaCO_3$.

BSA Release Study

While still immersed in pentane, three 20-cm sections were randomly selected and cut from each fiber type for use in release studies. The ends of the fibers were crimped using a hemostat, and then the sections were taken to an aseptic environment, removed from the pentane coagulant, and dipped for 10 s in 10× penicillin-streptomycin (P/S) solution to enhance sterility of the fiber surface. Upon removal from the P/S dip, the fiber sections were individually placed into appropriately labeled, sterile, 5-mL polypropylene tubes and temporarily capped. Once all fibers for the study had been so placed, all were covered completely with 3 mL of calcium-supplemented Tris buffer.

Immediately following immersion, the first assay time point was taken (time 0). This involved removal of the su-

pernatant from each vial, followed by its replacement with fresh release assay buffer. The supernatant was thoroughly mixed before removal from the assay vessel by repeatedly pipetting the solution while avoiding bubble generation. At completion of the last mixing cycle, the supernatant was extracted from the vessel in 1-mL increments and placed into three cuvettes for UV spectroscopic analysis.

The 3 mL of supernatant removed from each vessel was then replaced with 3 mL of fresh release assay buffer, and the vials containing the fibers and buffer were then tightly capped and placed into a tissue culture incubator at 37°C, 100% relative humidity, 5% CO_2 for storage until the next time point. At each time point, this sampling process was repeated for each fiber preparation.

Protein Determination

Alginates, even those purified to national formulary requirements, contain residual proteins.[30] Considering this fact, we determined total protein concentration in the supernatant samples for each fiber at each time point as a function of optical density at 281 nm after generating an appropriate calibration curve using BSA dilutions in assay buffer. At each time point, the three 1-mL supernatant samples from each fiber replicate were assayed vs. a release buffer blank in order to determine the concentration of total protein they contained. The fraction of total protein comprised of BSA originally contained within the alginate was calculated by subtracting the release data for the blank fiber that contained no added BSA, followed by statistical modeling of the release profiles.

Data Statistical Analysis

All data are reported as means ± 1 standard error (SE) for $n = 3$ for each fiber preparation and $n = 3$ for each sample. Cumulative protein release from the fiber replicates was analyzed through linear regression modeling, and the means of the coefficients of the second-order polynomial used to represent each data set were compared statistically to determine how each fiber treatment affected burst release relative to the others (the *y*-intercept of the model), and how the treatment affected the delivery of protein over time.

RESULTS AND DISCUSSION

The PLLA fiber extrusion techniques discussed in this article repeatably produce a hydrogel-cored bicomponent fiber with an outer diameter that is adjustable through the modification of extrusion parameters to the range of 200–500 μm. Figure 2 provides a scanning electron microscope (SEM) image of the sheath cross-section from a blended PLLA fiber formed as described in this article. As evident in Figure 2, the fibers exhibit marked porosity and include large voids created though the incorporation of an emulsion in the fiber.

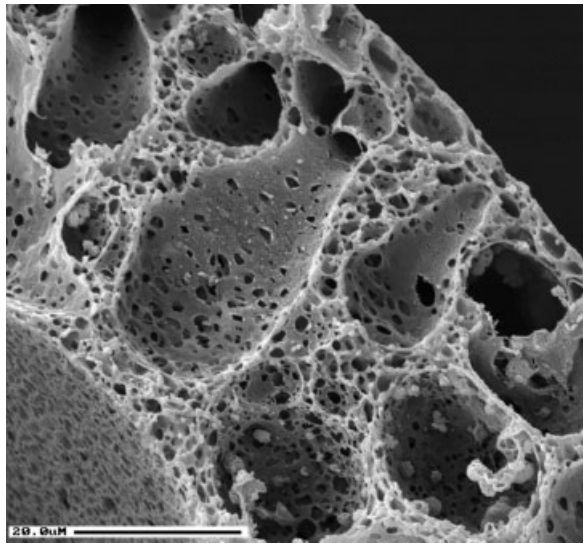

FIGURE 2 SEM taken at 1000× magnification, 15.0 kV, detailing the wall cross-section of a hydrogel-cored, PLLA blend fiber incorporating a 10% aqueous phase incorporated as an emulsion within the polymer shell. Bar represents 20 μm.

In Vitro Release of Total Protein from an Alginate-Cored PLLA Fiber

Total protein released from the fiber sections at each time point in the assay was determined from a calibration curve for optical density vs. protein concentration for BSA in solution detected at 281 nm. Each collected sample was analyzed spectrographically at this wavelength and blanked to assay buffer to yield a UV absorbance value for each time point.

Figure 3 illustrates the protein released from the four fiber treatments through the first two time points of the study (0 and 21.5 hours), as well as for the sum of the protein released for these two periods. When assessing this graphic, one should recall that alginates typically carry residual proteins that are not eliminated in their purification processes[30] ; therefore, detectable protein is shown in the figure for the blank fiber, which contained no loaded BSA.

With respect to the error bars depicted, representing ±1 SE for a mean of nine sampled volumes, there is no statistical difference in the first time point (0 h) burst release from the fibers containing 0 and 5% emulsions, while there is a difference between the 10% fiber and these two other BSA-containing fibers ($p < .00001$, $\alpha = 0.05$). Additionally, all BSA-loaded fibers show a statistically significant difference between them and the blank fiber, with emphasis on the 0% fiber, which contained BSA in its alginate core but no emulsion in the polymer. This indicates that the BSA load carried by these fibers significantly con-

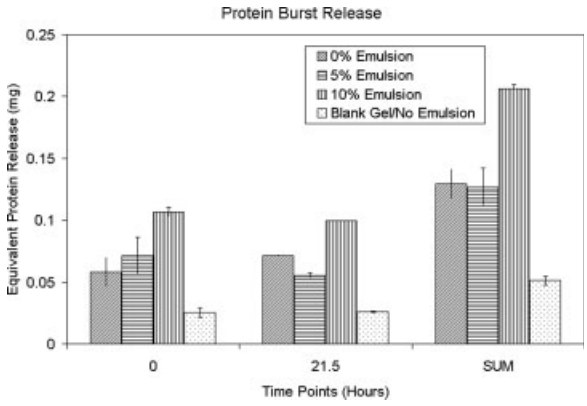

FIGURE 3 Burst release of total protein in equivalent BSA mass from 0%, 5%, 10% and blank PLLA fibers for time points 1 and 2 (0 and 21.5 h, respectively), with the summation of these points also provided. The blank fiber contains alginate in its core with no BSA loaded. The 0, 5, and 10% fibers contain loaded BSA in their cores, as well as the respective volume percentage of aqueous phase incorporated into emulsions in their polymer shells. The emulsions were created using DPPC as a surfactant.

tributed to the protein burst release from the fibers and that the burst is primarily this added protein rather than the protein intrinsic to the alginate.

The total protein release indicated for all fibers for the second time period (21.5 h) is statistically equivalent to that for the first period, though the standard errors are smaller for each fiber. Cumulative protein release over the first two time periods is statistically equivalent for the 0 and 5% BSA-loaded fibers. The summation of these two times emphasizes the significant difference in the release from the 10% emulsion fiber when compared to the other treatments. When compared to the blank fiber, the protein release quantities shown for the loaded fibers again emphasize the impact the added BSA has on the total protein burst release.

The contribution of the added BSA to the total protein burst release is evident in the graphs for the three loaded fibers. The magnitude of this contribution is clarified by comparison of the protein release from the 0% emulsion fiber and the blank fiber. The blank fiber only contained proteins intrinsic to the stock alginate, while the 0% emulsion fiber contained the identical volume of alginate (hence, intrinsic protein) loaded with BSA at 0.5 wt %. Hence, subtracting the observed total protein quantity released from the blank fiber from that of the 0% fiber indicates the approximate contribution to the release from the loaded BSA. In this case, the blank fiber released approximately 0.05 mg of total protein (no BSA), while the 0% emulsion fiber released approximately 0.13 mg of total protein (intrinsic proteins plus BSA). Therefore, the BSA loaded into the core of the 0% emulsion fiber contributed approximately 0.08 mg to the total protein release. Increasing the aqueous phase concentration of the emulsion to 10% increased total protein burst, but the effect on the release of the BSA component alone cannot be determined using the same comparison.

The cumulative in vitro total protein release behavior of the various fiber treatments studied is graphically depicted in Figure 4. The values provided are for equivalent milligrams of BSA vs. time in days and were determined by summing the time point release data. As evident from the graph, fibers containing emulsions generally exhibited respectively greater total release throughout the study, though release from the 0 and 5% emulsion fibers overtakes that of the 10% fiber at the end of the investigation. The cumulative profiles for all fiber treatments follow virtually a linear path. Overall, the profiles for the 0 and 5% emulsion fibers track almost identically throughout the study, while cumulative release from the 10% emulsion fiber exceeds that of all other fibers except for the final time point.

In order to statistically analyze cumulative in vitro protein release behavior of the various fiber treatments, the total protein release data for all time points were first modeled mathematically using an automated curve fitting and equation discovery program (SYSTAT Software TableCurve 2D, version 5.01). The curve-fitting engine of this program assessed the cumulative release data for each fiber type, and based on the resulting fit equations, a single mathematical

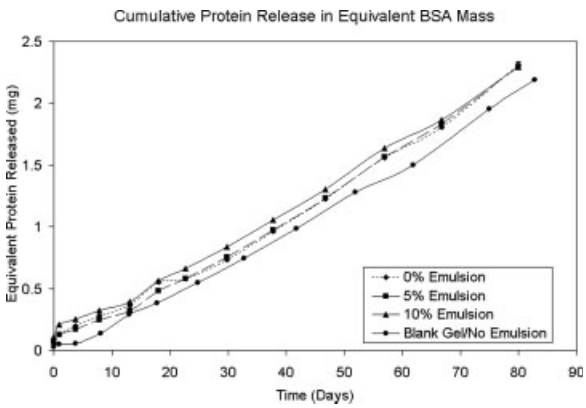

FIGURE 4 Cumulative total protein released from all fiber treatments in equivalent milligrams (mg) BSA. The blank fiber contains alginate in its core with no BSA loaded. The 0, 5, and 10% fibers contain loaded BSA in their cores, as well as the respective volume percentage of aqueous phase incorporated into emulsions in their polymer shells. The emulsions were created using DPPC as a surfactant.

model was then user selected to represent the release profile for each treatment. Though, graphically, the cumulative protein release data depicted in Figure 4 appears close to linear and of the form $y = b + mx$, a two-parameter equation of the form $y = a + b \cdot xlnx$, linear in its parameters, better approximated the data. Selection of this equation as the best model representing all fiber release data was based on the graph of the model vs. the observed data, the lack of outliers, the normality of residuals, the confidence intervals of the predicted data, and the additional fit parameters weighted by the program. For the selected model, the coefficients of determination for each fiber treatment were greater than $r^2 = 0.997$ and the F statistics were all above 4200. Additionally, each parameter defining the mathematical model for each fiber treatment was statistically significant to the model, with $\alpha = 0.05$.

Statistical analysis of the model's parameters for cumulative protein release indicates that all intercept parameters (the ''a'' parameters) are significantly different from zero, even for the blank fiber. This fact can be interpreted as statistical verification that the initial release from the BSA-loaded fibers also consists of protein from the alginate. The composition of the protein contained within the stock alginate has not yet been determined in our laboratory.

Additional analysis of intercept parameters for all the fibers via inspection of their confidence intervals indicates that each of the BSA-loaded fibers has a burst release that is statistically different from that of the blank, regardless of its emulsion load. Therefore, the BSA loaded into the fiber is a significant contributor to the overall protein burst release from these fibers. Comparison of the 95% confidence intervals for the intercept parameters for these BSA-loaded fibers also indicates a significant statistical difference between the 10% fiber and both the 0 and 5% fibers, while the 5 and 0% fibers do not differ statistically in their intercept parameters. This corresponds well with the actual burst release data shown in Figure 3.

In order to determine if the various fiber types give statistically significant differences in the overall cumulative protein release profiles, 95% confidence intervals were analyzed for each fiber type throughout the time course of the experiment. Analysis indicated there is no overlap in the 95% confidence intervals between the blank fiber (no BSA load) and any of the other fiber treatments examined. Therefore, the additional protein loaded into the remaining fibers contributed significantly to their overall cumulative protein release profiles.

In order to determine the effect of emulsion loading on the cumulative protein release profiles, the 95% confidence intervals for predicted data for the fiber treatments containing additional BSA and 0, 5, and 10% emulsions were compared statistically. Analysis indicates that the protein delivered from the 5 and 10% emulsion-loaded fibers is statistically different through the 67th day of the study, while the release profiles for the 0 and 5% fibers are statistically equivalent through the entire course of the study. Additionally, the 0 and 10% fibers become statistically equivalent near the 47th day of the experiment. These differences are based on the model selected to represent the observed cumulative release data and are in good agreement with the variances depicted in the plot of the actual data (Figure 4).

The fact that the blank contained protein from the alginate is often overlooked in studies of protein released from alginate gels or beads, and can lead to misinterpretation of results, especially when general protein detection assays are employed (such as Bradford).

As noted in Figure 4, total protein release from the blank fiber treatment was assayed at different time points than the other fibers after the third point (3.75 days). Hence, the quantity of BSA released into the supernatant from the loaded fibers could not be determined directly from protein release data, nor from a simple subtraction of the blank fiber data from each BSA-loaded fiber each time point (due to the sampling time variation). The determination of the release profile for the BSA loaded into the alginate cores was only made possible by modeling the cumulative protein release from all the fibers and comparing the mathematical models of release for the BSA-loaded and blank fibers at equivalent time points.

In Vitro Release of BSA from an Alginate-Cored PLLA Fiber

Using the models created for statistical study of the total protein release from the fiber treatments, single protein release (BSA) was determined through the mathematical evaluation of the protein release data. This evaluation assumes accuracy of the models (which all had r^2 values greater than 0.997 and high F statistics), and equivalence of the residual protein quantity contained within the alginate cores of each fiber. Residual protein should have been equivalent because the alginate used for all fibers was from the same source, and the fibers theoretically contained the same volume of gel.

Tests in our laboratory (nonpublished data) have indicated that fibers of the construction cited in this article consistently carry 100 μL of gel per 33 cm of extruded fiber for the polymer and hydrogel flow

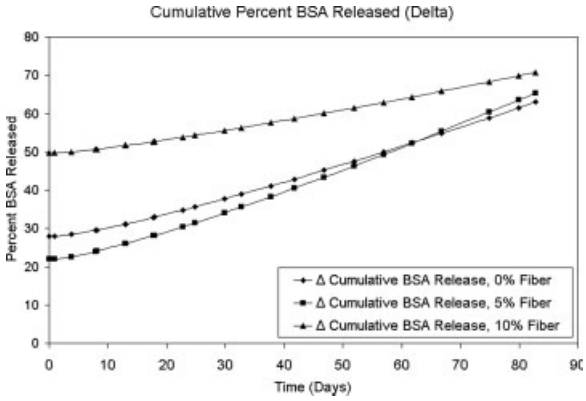

FIGURE 5 Cumulative BSA release expressed in percent of initial load. Theoretical initial BSA load was 303 μg. Mathematical analysis of modeled release data yielded the percentages indicated.

rates provided. Based on this data, and assuming no losses of BSA into the coagulation bath during the extrusion process, each 20-cm, nonblank fiber replicate studied carried approximately 60.6 μL of alginate gel loaded with 303 μg of BSA within its core.

This BSA load was used to calculate the cumulative percentage protein delivered for each fiber type as indicated in Figure 5. Data points for this graphic were generated from mathematical comparison to the release data from the blank fiber, expressed in percent initial load. As indicated, the 10% emulsion fiber released the greatest amount of BSA over the course of the study, approximately 70%, while the 0 and 5% fibers released approximately the same total (63 and 65% of the BSA, respectively). More critical to the overall release profile is the fact that the 10% fiber released nearly 50% of its hypothetical BSA load by the first assay time point, which was approximately double the initial release of the 0% emulsion fiber.

This high burst release and the respective difference to that from the 0% emulsion fiber is likely due the high degree of porosity and the level of macrovoid formation in the 10% emulsion fiber. As indicated in Figure 2, the porosity within the wall (shell) of the fiber carries though from the center bore to the surface, with likely interconnections or channels throughout. Such porosity would greatly enhance the diffusion of BSA loaded in the hydrogel core of the fiber to the surface of the fiber and out into the release media. Burst release is approximately 28% of the total BSA load for the 0% fiber treatment, and 22% for the 5% treatment. These release percentages indicate that the emulsion within the polymer contributes significantly to the burst release from the fiber, especially for the highest aqueous load tested. An artifact of the modeling process makes it appear that the burst from the 0% emulsion fiber exceeded that for the 5% fiber, but actual data does not support this. These values correspond well to the cumulative total protein release profiles and the statistical differences previously noted for the various fiber treatments.

Statistical analysis of the delta protein models has indicated a significant difference between the model parameters of all three BSA-loaded fibers. The intercept parameters for the models are significantly different from zero and are all significantly different from each other. The modeled release profile for the 10% fiber is significantly different from that of the 0 and 5% fibers through the course of the study, and these two treatments are significantly different at all time points except when their profiles converge near day 62. Overall, the delta BSA models indicate that the 10% emulsion, BSA-loaded fiber released more BSA than the 0 and 5% fibers, and the 5% fiber ultimately (from the last three data points) released slightly more of its BSA load than did the 0% fiber.

Influence of Emulsion Load on Extrusion

As noted, the incorporation of an emulsion within the fibers resulted in greater initial burst release of protein, with more protein released as the emulsion concentration increased. This burst release contributes significantly to the cumulative release of protein from the fibers, such that the greater the emulsion load, the greater the cumulative release. Therefore, burst release is a condition that must be rectified to better control drug or protein release from these fibers. However, much experience in the development of this fiber construction has shown that adding an emulsion to the polymer improves the extrusion process and tends to allow the formation of a more consistent fiber (unpublished data). Therefore, the benefit of loading some level of emulsion into the polymer possibly outweighs the detrimental effect on burst release. Considering the significant difference in burst release noticed between the 5 and 10% fibers, an emulsion load less than 5% would be a good choice for future investigations.

CONCLUSION

This work addressed the assay of a novel, hydrogel-cored PLLA fiber to deliver a model protein in an in vitro environment. Our investigation indicated that a 10% aqueous emulsion in a PLLA sheath led to greater burst and overall release than did the 0 or 5% aqueous emulsion fibers. A single polymer composition containing various emulsion levels was studied,

along with a single alginate composition within the cores of the fibers. Hence, this investigation only addressed the affect of the emulsion load on the release of the model protein.

Considering that the composition of an alginate gel affects its porosity and strength,[23,31–33] a continuation of this investigation would be to examine the effect of modifications to the alginate on the release profile of the contained drug or protein. Additionally, this study examined a blend of PLLA in order to accelerate the delivery of BSA from the gel. Considering that lactate is a chelating agent for calcium and that the degradation of sodium alginate crosslinked with Ca^{2+} ions can occur by removal of the calcium,[14] it would be interesting to examine the effect on the alginate core by extruding in a faster-degrading polymer like poly(DL-lactic-*co*-glycolic acid) (PLGA). Degradation of PLGA results in the formation of lactic acid, which will act as a chelating agent for the Ca^{2+} ions used in formation of the alginate gel. Availability of lactic acid in proximity to the gel should result in its degradation through the removal of calcium. Thus, degradation of the hydrogel can be controlled by the degradation of the PLGA. This can lead to leakage of entrapped material, which should increase the drug delivery rate.

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REFERENCES

1. Schwab, M. E.; Bartholdi, D. Physiol Rev 1996, 76, 319–370.
2. Rangappa, N.; Romero, A.; Nelson, K. D.; Eberhart, R. C.; Smith, G. M. J Biomed Mater Res 2000, 51, 625–634.
3. Lieberman, A. R. Brain Res Bull 1999, 50, 401–402.
4. Fawcett, J. W.; Asher, R. A. Brain Res Bull 1999, 49, 377–391.
5. Aldini, N. N.; Perego, G.; Cella, G. D.; Maltarello, M. C.; Fini, M.; Rocca, M.; Giardino, R. Biomaterials 1996, 17, 959–962.
6. Seckel, B. R.; Chiu, T. H.; Nyilas, E.; Sidman, R. L. Plast Reconstr Surg 1984, 74, 173–181.
7. den Dunnen, W. F.; Stokroos, I.; Blaauw, E. H.; Holwerda, A.; Pennings, A. J.; Robinson, P. H.; Schakenraad, J. M. J Biomed Mater Res 1996, 31, 105–115.
8. den Dunnen, W. F.; van der Lei B.; Schakenraad, J. M.; Stokroos, I.; Blaauw, E.; Bartels, H.; Pennings, A. J.; Robinson, P. H. Microsurgery 1996, 17, 348–357.
9. Widmer, M. S.; Gupta, P. K.; Lu, L.; Meszlenyi, R. K.; Evans, G. R.; Brandt, K.; Savel, T.; Gurlek, A.; Patrick, C. W., Jr.; Mikos, A. G. Biomaterials 1998, 19, 1945–1955.
10. Nelson, K. D.; Romero-Sanchez, A.; Alikacem, N.; Smith, G. M.; Waggoner, P.; Radulescu, D.; Hu, Z. U.S, Patent Number 6,596,296, 2003.
11. Nelson, K. D.; Romero, A.; Waggoner, P.; Crow, B.; Borneman, A.; Smith, G. M. Tissue Eng 2003, 9, 1323–1330.
12. Crow, B. B.; Borneman, A. F.; Hawkins, D. L.; Smith, G. M.; Nelson, K. D. Tissue Eng 2005, 11, 1077–1084.
13. Stockwell, A. F.; Davis, S. S.; Walker, S. E. J Control Release 1986, 3, 167–175.
14. Gombotz, W. R.; Wee, S. Adv Drug Del Rev 1998, 31, 267–285.
15. Mumper, R. J.; Huffman, A. S.; Puolakkainen, P. A.; Bouchard, L. S.; Gombotz, W. R. J Control Release 1994, 30, 241–251.
16. Kuo, C. K.; Ma, P. X. Biomaterials 2001, 22, 511–521.
17. Mosahebi, A.; Simon, M.; Wiberg, M.; Terenghi, G. Tissue Eng 2001, 7, 525–534.
18. Kataoka, K.; Suzuki, Y.; Kitada, M.; Hashimoto, T.; Chou, H.; Bai, H.; Ohta, M.; Wu, S.; Suzuki, K.; Ide, C. Tissue Eng 2004, 10, 493–504.
19. Kataoka, K.; Suzuki, Y.; Kitada, M.; Ohnishi, K.; Suzuki, K.; Tanihara, M.; Ide, C.; Endo, K.; Nishimura, Y. J Biomed Mater Res 2001, 54, 373–384.
20. Suzuki, Y.; Kataoka, K.; Kitada, M.; Wu, S.; Hashimoto, T.; Ohnishi, K.; Suzuki, K.; Ide, C.; Endo, K.; Nishimura, Y.; Tanihara, M. Eur J Neurosci 2000, 12, 287.
21. Nelson, K. D.; Crow, B. B. U.S, Patent Application Number 10/428,901, 2004.
22. Draget, K. I.; Skjak-Braek, G.; Smidsrod, O. Int J Biol Macromol 1997, 21, 47–55.
23. Draget, K. I.; Skjak Braek, G.; Smidsrod, O. Carb Polym 1994, 25, 31–38.
24. Shchipunov, Y. A.; Koneva, E. L.; Postnova, I. V. Polym Sci Ser A 2002, 44, 758–766.
25. Draget, K. I.; Ostgaard, K.; Smidsrod, O. Carb Polym 1990, 14, 159–178.
26. Xu, X.; Yu, H.; Gao, S.; Ma, H. Q.; Leong, K. W.; Wang, S. Biomaterials 2002, 23, 3765–3772.
27. Sanchez, A.; Villamayor, B.; Guo, Y.; McIver, J.; Alonso, M. J. Int J Pharmaceutics 1999, 185, 255–266.
28. Reich, G. Eur J Pharm Biopharm 1998, 45, 165–171.
29. Igartua, M.; Hernandez, R. M.; Esquisabel, A.; Gascon, A. R.; Calvo, M. B.; Pedraz, J. L. Int J Pharmaceutics 1998, 169, 45–54.
30. De Vos, P.; De Haan, B. J.; Wolters, G. H.; Strubbe, J. H.; Van Schilfgaarde, R. Diabetologia 1997, 40, 262–270.
31. De Vos, P.; De Haan, B.; Van Schilfgaarde, R. Biomaterials 1997, 18, 273–278.
32. Draget, K. I.; Simensen, M. K.; Onsoyen, E.; Smidsrod, O. Hydrobiologia 1993, 261, 563–569.
33. Becker, T. A.; Kipke, D. R.; Brandon, T. J Biomed Mater Res 2001, 54, 76–86.

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