The Effect of Additives on the Behavior of Phase Sensitive *In Situ* Forming Implants

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ABSTRACT: Phase-sensitive *in situ* forming implants (ISFI) are a promising platform for the controlled release of therapeutic agents. The simple manufacturing, ease of placement, and diverse payload capacity make these implants an appealing delivery system for a wide range of applications. Tailoring the release profile is paramount for effective treatment of disease. In this study, three innovative formulation modifications were used to control drug release. Specifically, water, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI), and bovine serum albumin (BSA) were incorporated into an ISFI solution containing the small molecular weight mock drug, sodium fluorescein. The effects of these additives on drug release, swelling, phase inversion, erosion, and implant microstructure were evaluated. Diagnostic ultrasound was used to monitor changes in swelling and phase inversion over time noninvasively. Water, DiI, and the combination of BSA/DiI functioned to reduce burst release 47.6%, 76.6%, and 59.0%, respectively. Incorporation of water into the casting solution also enhanced the release of drug during the diffusion period of release by 165.2% relative to the excipient free control. Incorporation of BSA into the polymer solution did not significantly alter the burst release ($p < 0.05$); however, the onset of degradation facilitated release was delayed relative to the excipient-free control by 5 days. This study demonstrates that the use of excipients provides a facile method to tailor the release profile and degradation rate of implants without changing the polymer or solvent used in the implant formulation, providing fine control of drug dissolution during distinct phases of release. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:3471–3480, 2015

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INTRODUCTION

Phase-sensitive *in situ* forming systems have been used for fabrication of numerous products ranging from asymmetric membranes for reverse osmosis filtration to drug eluting polymer depots for the treatment of prostate cancer.[1–7] These phase-sensitive systems exist as liquid polymer solutions until introduced to a nonsolvent, such as water, where the polymer precipitates into a solid matrix through a process of phase inversion.[5,8–11] In medical applications, phase-sensitive *in situ* forming implants (ISFI) are of interest because they provide a platform by which drug can be delivered through a minimally invasive injection, which facilitates accurate placement of the implant in a desired location for the controlled local delivery of drug over time.[12] These implants are manufactured by dissolving a biodegradable polymer into a biocompatible solvent, which can be mixed with a drug to form a suspension or solution.[13,14] During phase inversion, the nonsolvent diffuses into the polymer solution, whereas the solvent diffuses out of the implant into the surrounding environment resulting in an unstable ternary system until sufficient nonsolvent can induce precipitation of the polymer.[5,6] A number of systems have been used to characterize the phase inversion process such

as dark ground imaging, electron spin resonance, and ultrasound imaging.[10,15–18] Ultrasound imaging works by detecting the changes in acoustic impedance that occur as the polymer precipitates out of solution.[6,17] It is the only method that allows for the visualization of the process both *in vitro* and *in vivo*, and can be used to detect other processes such as implant swelling and erosion.[14,19]

Phase-sensitive ISFIs have a release profile with three distinct phases.[20] Burst release occurs over the course of the first 24–48 h and is identified as the initial rapid release of drug from the implant. Diffusion-facilitated release occurs as the drug moves through the polymer matrix and is controlled by the implant microstructure.[21–23] Degradation-facilitated release occurs as a result of polymer degradation increasing the interconnectivity of the porous microstructure of the implant and results in an increase in the release of drug. A number of methods have been used to control the release profile of phase-sensitive ISFIs, typically by altering the mass transfer kinetics of the solvent/nonsolvent in order to increase or decrease the porosity of the polymer matrix.[8,21,23–25] Factors such as changes in the polymer molecular weight (MW), concentration of the casting solution, or the solvent composition have all been successfully used to change the release profile of the implant.[16,19,22,26–30] However, these changes often result in an increased solution viscosity that can limit the injectability of the solution.[31] The use of additives provides a means to alter distinct phases of the release profile of phase-sensitive ISFIs, and tailor the release

profile for a specific application, without increasing the solution viscosity.

In the present study, the effect of three unconventional additives on the release profile from ISFIs was evaluated. These excipients function as agents added to the polymer formulation solution to enhance the function of the implants, without inducing a therapeutic effect.[32] Water was incorporated into the casting solution in order to change the solvent/nonsolvent mass transfer kinetics. 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) has been shown to increase the hydrophobicity of the polymer solution and consequently reduce the interconnectivity of the internal porous network,[14] and was selected to reduce both burst and diffusion-facilitated release. Bovine serum albumin (BSA) has been shown to reduce the erosion of ISFIs, and was introduced to alter the onset of degradation-facilitated release.[14] Finally, a combination of BSA/DiI was used to evaluate the combinatorial effect of additives on the release profile. Changes in drug release were evaluated by measuring the dissolution kinetics of the mock drug sodium fluorescein (MW = 376, log $P = -1.52$, pKa = 6.4).[14,33] Sodium fluorescein was specifically chosen because it is a small molecular weight hydrophilic agent that has a release profile with distinct regions of release following a pattern of burst, diffusion, and degradation-facilitated release. Furthermore, this mock drug also has a release profile similar to that observed with the therapeutic agent doxorubicin.[14] Implant erosion and fluid uptake were measured by monitoring changes in the implant mass. Polymer phase inversion was evaluated using ultrasound imaging, and the implant microstructure was evaluated using scanning electron microscopy (SEM). Results from this study will provide insight into additional methods for controlling the release profile of ISFIs, providing control over the dissolution and degradation kinetics of drugs during each distinct phase of release.

MATERIALS AND METHODS

Materials

Poly(DL-lactic-*co*-glycolic acid) (PLGA; 50:50, MW 21,000 Da) was obtained from Evonik Industries (Rellinghauser Strasse, Germany) and used as received. N-methyl-2-pyrrolidinone (NMP), sodium fluorescein, and BSA were used as received from Sigma–Aldrich (St. Louis, Missouri). DiI was used as received from Advancing Assay and Test Technologies (Sunnyvale, California). Agarose and phosphate-buffered saline (PBS) were received from Fischer Scientific (Waltham, Massachusetts).

Polymer Solution Formulations

Control solutions with no additives were prepared using a 39:60:1 mass ratio of PLGA–NMP–fluorescein by first suspending or dissolving the fluorescein in NMP as previously described.[14,19] One hour before injection into the bath solution, BSA was added to the caste solution (35:60:4:1 mass ratio of PLGA:NMP:BSA:fluorescein), and allowed to mix using a magnetic stirrer. The solutions were then warmed to 37°C in order to eliminate air bubbles in the solution. Solutions containing DiI (35:60:4:1 mass ratio of PLGA:NMP:DiI:fluorescein) were prepared by first dissolving the fluorescein and DiI in NMP, followed by addition of the PLGA, which was stirred overnight in a 37°C shaker table at 90 rpm. Solutions containing both DiI and BSA (BSA/DiI) (35:60:2:2:1 mass

ratio of PLGA:NMP:BSA:DiI:fluorescein) were prepared by first dissolving the fluorescein and DiI in NMP, then adding PLGA and stirred overnight, with BSA added as described earlier. Solutions containing water (39:54:6:1 mass ratio of PLGA:NMP:water:fluorescein) were prepared similar to control solutions, but a 10% water in NMP solution was used instead of pure NMP. Polymer solutions with water were prepared the day before use; all other formulations were stored at 4°C and used within 3 days.

Cumulative Drug Release

Drug release profiles were evaluated as described previously.[14] First, polymer solution (42.9 ± 5.2 mg) was injected into 10 mL of 37°C PBS (pH 7.4), and then placed in an incubated orbital shaker (37°C at 90 rpm). Over the course of the first 6 h, 1 mL of solution was sampled and then replaced with 1 mL of fresh warm PBS at predetermined time points (0, 0.5, 1, 2, 4, and 6 h after injection into PBS). After 24 h, a sample was taken, and then the bath solution was completely removed and replaced by 10 mL of fresh buffer daily for 14 days. After 14 days, implants were removed from the bath solution and degraded in 5 mL of 2M NaOH. Fluorescein mass was determined by measuring the fluorescence in the solution samples that was compared with a standard curve of known fluorescein concentrations using a multimode microplate reader (Infinite 200 series; Tecan Ltd.; Männedorf, Zürich, Switzerland) at excitation/emission wavelengths of 485/525 nm. Cumulative drug release was calculated from these measurements and normalized by the total mass of drug in the implant.

Implant Imaging

Implants were imaged using diagnostic ultrasound as previously described.[17] Briefly, implants were imaged through the Z-axis by immobilizing a 12-MHz transducer (Aplio XG; Toshiba Medical Systems; Minato, Tokyo, Japan) below an agarose mold containing the implant. Images were taken at predetermined time points over the course of the first 24 h (0, 0.5, 1, 2, 4, 6, and 24 h after the implants were added to PBS), and then imaged daily until the study was terminated. The buffer solution was replaced daily after the images were taken. Implants were maintained at 37°C at 90 rpm for the duration of the study. Image analysis was performed to monitor changes in the polymer shell thickness by using a custom MatLab code as previously described (MathWorks Inc., Natick, Massachusetts).[17,34] First, a threshold was applied to the image in order to create a binary mask, which was normalized by the sum of the total number of pixels within the cross-sectional area of the implant.[17,34] Changes in the implant cross-sectional area over time were used as a metric of the implant swelling.[17,34]

Erosion and Bathside Uptake

Changes in implant mass with respect to time were monitored as describe previously.[14,19] First, the initial mass of implants added to 10 mL of warm PBS (pH 7.4) was recorded and then kept in an incubated shaker (37°C at 90 rpm). Then, implants were removed from the bath solution at predefined time points (1, 3, 7, 10, 14, 17, and 21 days after the polymer solution was added to PBS). At each time point, implants were dried with a clean paper towel, and then weighed in order to obtain the wet mass. The implants were then frozen, followed by lyophilization for 4 days. After lyophilization, the implant dry mass was
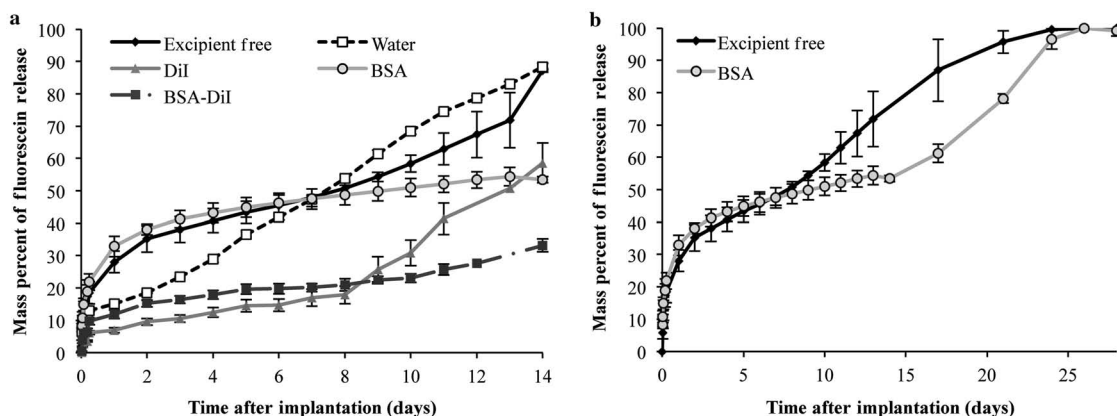
Figure 1. Cumulative release of sodium fluorescein over the course of 14 days (a) and 28 days (b). All error bars represent standard deviation with $n = 3$.

recorded. Fluid uptake was calculated by subtracting the wet mass from the initial mass then normalizing by the initial implant mass. Polymer erosion was determined by normalizing the implant dry mass with the theoretical polymer mass of the implant. Sink conditions were maintained by replacing the total volume of the buffer solution daily.

Scanning Electron Microscope Imaging and Analysis

Implant microstructure was evaluated as previously described.[14] Briefly, the implants were frozen and then fractured over dry ice. After the implants were freeze-fractured, the samples were lyophilized for 4 days. Next, the implants were mounted on an aluminum stub using carbon tape, and sputter coated with 5 nm of Pd. The coated samples were then imaged using a Quanta 200 3D ESEM (Hillsboro, OR) with an acceleration voltage of 3.5 kV and a hole size of 10.

Statistical Analysis

Statistical significance was performed using Minitab (Minitab Inc., State College, Pennsylvania). One-way analysis of variance (ANOVA, $p < 0.05$) was used to determine statistical significance, and a Tukey multicomparison test was used to compare differences between groups. All data were reported as mean ± standard deviation.

RESULTS

Cumulative Drug Release

In situ forming implants have characteristic release kinetics consisting of three distinct phases of release: burst, diffusion, and degradation. Burst release occurred over the course of the first 24 h after injection into the bathside solution. No significant difference in burst release was observed between implants formulated without additives and those containing BSA (28.8 ± 3.0% and 32.9 ± 3.2%); however, these implant formulations did have a significantly greater burst release than all other depot formulations (Fig. 1). The additives water, DiI, and BSA/DiI reduced the average burst release by 47.6%, 75.9%, and 58.8%, respectively.

Table 1. Average Release of Mock Drug at Each Phase of Release

	Burst (%)	Diffusion (%/day)	Degradation (%/day)
Excipient free	28.8 ± 3.0	2.3 ± 0.2	4.5 ± 1.7
Water	15.1 ± 0.3	6.1 ± 0.1	–
DiI	6.9 ± 0.8	1.5 ± 0.3	6.5 ± 1.0
BSA	32.9 ± 3.2	1.3 ± 0.1	4.4 ± 0.3
BSA–DiI	11.8 ± 1.3	1.0 ± 0.1	2.0 ± 0.3

The diffusion period of release occurred after 24 h, and the onset of degradation-facilitated release was determined by evaluating the derivative of the release plots.[19] Implants that used water as an additive had a significantly greater release rate than all other implant formulations (6.1 ± 0.1%/day). The addition of BSA or DiI into the polymer solution significantly reduced the daily release rate of mock drug from the implants (1.3 ± 0.1%/day and 1.5 ± 0.3%/day for BSA and DiI, respectively) compared with implants that contained no additives (2.3 ± 0.2%/day). The greatest decrease in diffusion-facilitated release was observed using a combination of BSA/DiI in the depot, resulting in a release rate of 1.0 ± 0.1%/day.

Degradation-facilitated release was not observed when water was used as the additive (Fig. 1). However, a significant increase in the drug dissolution rate was observed for all other implant formulations. The use of DiI and BSA/DiI did not alter the onset of degradation-facilitated release, but incorporation of BSA alone delayed degradation facilitated release for 5 days. The use of BSA or DiI as standalone additives did not significantly alter the daily dissolution kinetics of mock drug during degradation-facilitated release. However, significantly lower dissolution kinetics was observed from implants that contained the combination of BSA/DiI. The release rate results are summarized in Table 1.

Ultrasound Characterization

Implant Morphology

Representative gray-scale images of the implant cross-section over time are shown in Figure 2. Implants without an additive developed a thin shell immediately after injection into the
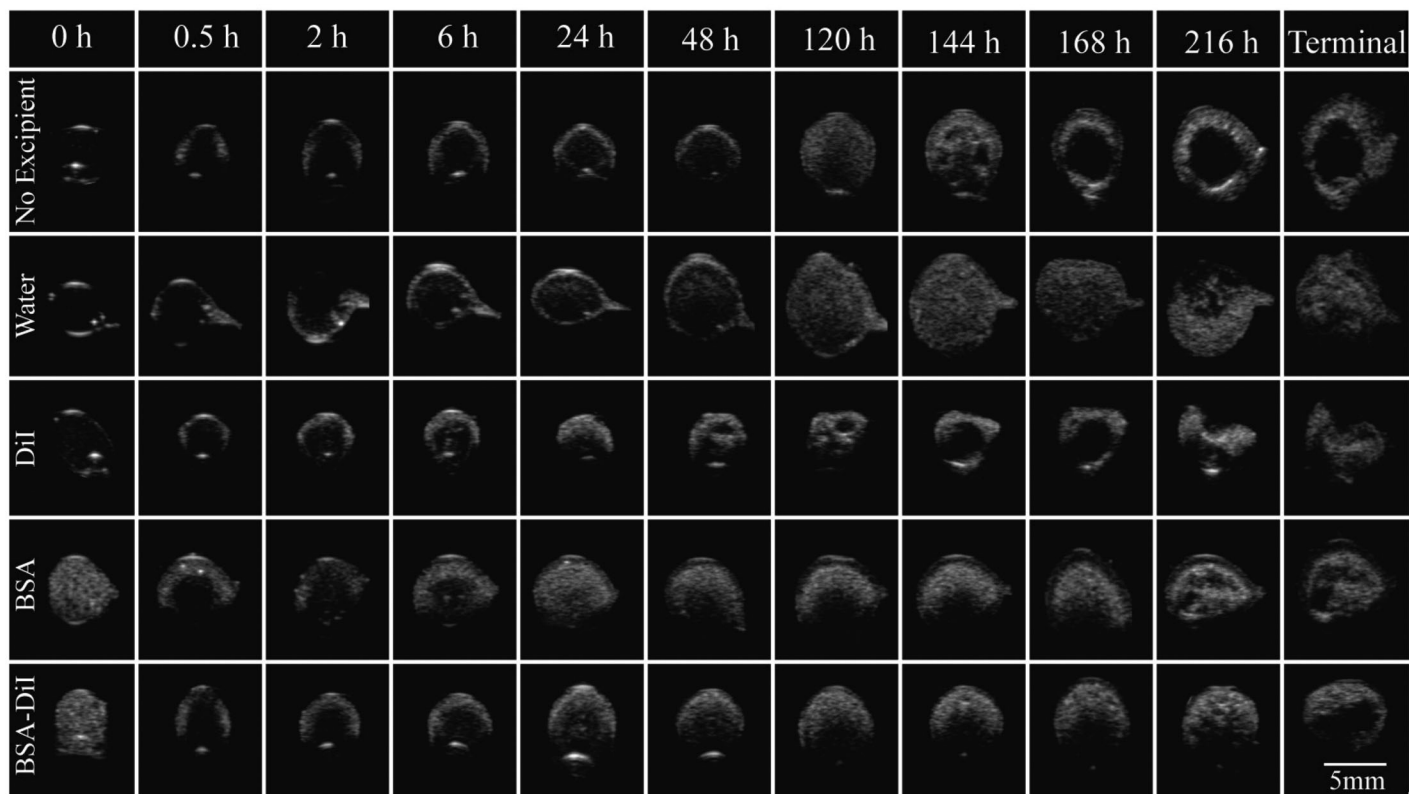
Figure 2. Representative ultrasound images of the same implant over the course of the study. Polymer precipitation results in white, whereas darker areas suggest a polymer lean domain. Row 1 is an excipient-free control, row 2 contains water in the casting solution, row 3 contains DiI, row 4 contains BSA, and row 5 is a combination of BSA/DiI.
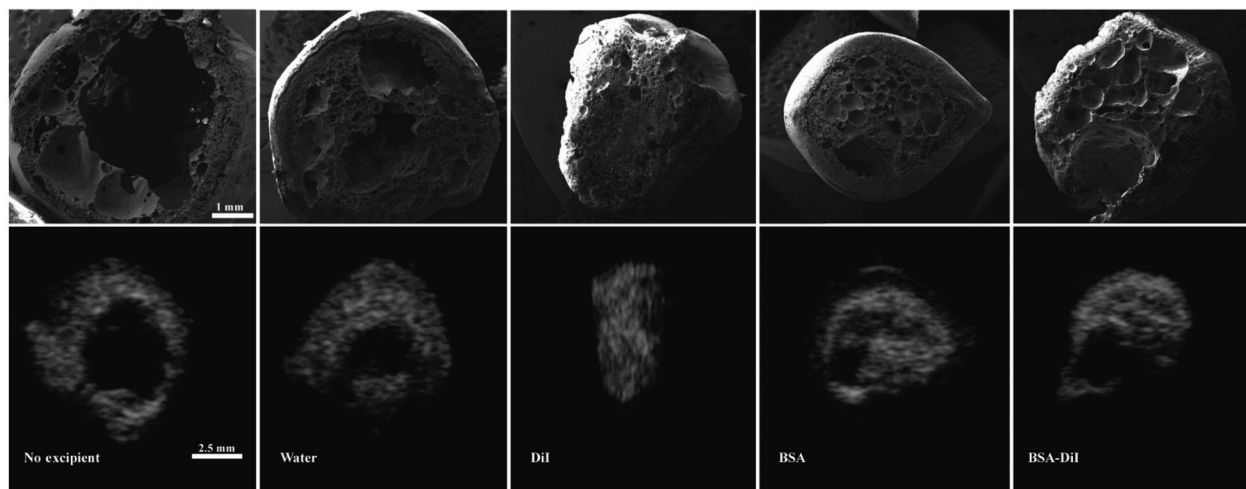

Figure 3. Comparison of implant morphology obtained using SEM with those obtained from ultrasound.

bathside. Over time, an increase in hyperechoic regions were observed as the polymer precipitated. After 6 days in the nonsolvent, large pores began to form in the center of the implant, which increased in diameter over the duration of the study. When water was used as the additive, implant behavior followed a similar pattern of formation. However, the development of the central pore did not occur until after 9 days in the bathside, and the pore diameter did not expand (Fig. 2). Implants formulated with DiI precipitated more rapidly than those without additives; additionally, the formation of the central pore occurred after 5 days followed by the implant collapsing after 9 days (Fig. 2). Implants that contained BSA had a high initial echogenic signal. This elevated echogenicity was lost within the first half hour, followed by the gradual increase in backscatter over the course of the first 24 h. Development of an interior pore did not occur until after 9 days in the nonsolvent, and the pore did not expand during the study. Implants with the combination of BSA/DiI had an initial elevated echogenic signal similar to implants with BSA alone, but the central pore did not occur until after 10 days. Image validation was performed by
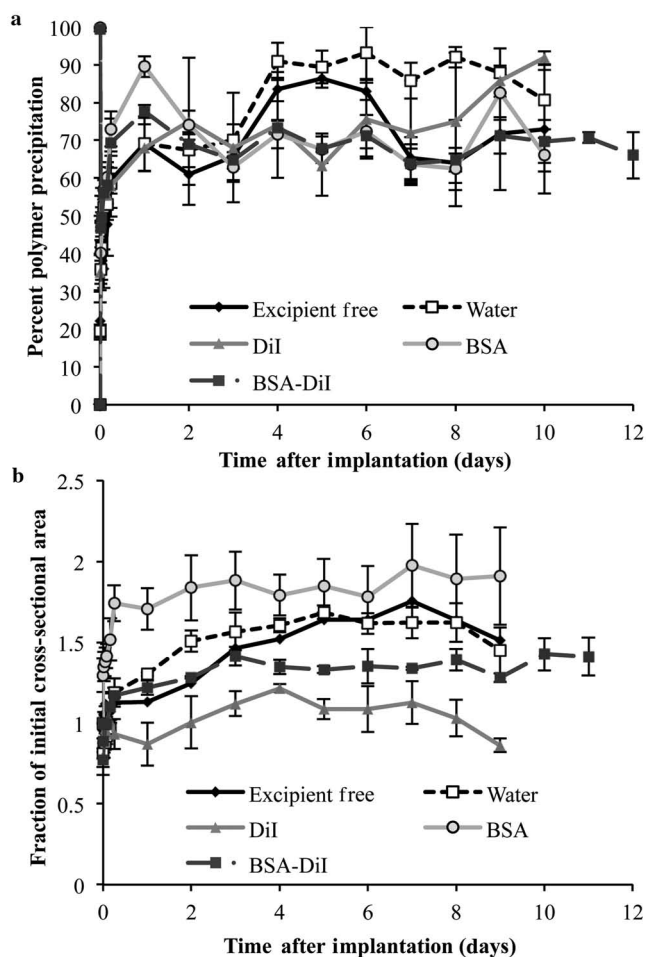
Figure 4. Quantitative formation data of ISFIs (a) and the change in the implant cross-sectional area over time (b). All error bars represent standard deviation with $n = 3$.

comparing the ultrasound images to SEM images of cryosectioned implants, demonstrating that pore formation observed using ultrasound imaging could be observed in the implants for all polymer solution formulations (Fig. 3).

Phase Inversion

Initial polymer precipitation occurred rapidly, reaching $69.1 \pm 7.3\%$ after 1 day for implants with no additives. These implants then continued to phase invert more slowly, with a peak polymer precipitation occurring after 4 days lasting through 6 days. After 6 days, a loss of signal was observed because of the formation of the central pore, which resulted in a decrease in the precipitated area. When water was used as the excipient, peak polymer precipitation occurred after 4 days, with no significant loss in signal occurring throughout the remainder of the study (Fig. 4a). Implants containing DiI rapidly precipitated over the course of the first 2 h, with no significant changes occurring until after 6 h, where more gradual precipitation occurred until reaching a peak after 2 days. After 5 days, a loss of signal was observed as a result of the formation of the pore. Implants with BSA and BSA/DiI both reached a maximum polymer precipitation after 1 day. A reduction in backscatter occurred after 1 day, but was not the result of a pore.

Implant Swelling

Implants without excipient initially shrink, and returned to the initial cross-sectional area after 4 h. The implants then continued to swell, reaching a maximum of 1.64-fold the initial cross-sectional area after 6 days (Fig. 4b). Implants using water as the excipient followed a similar pattern of swelling. Peak swelling occurred after 6 days, and reached a maximum of 1.69 ± 0.04-fold more than the initial cross-sectional area. Implants that contained DiI initially shrink and then reached a maximum cross-sectional area after 2 days in PBS (1.22 ± 0.03-fold the initial area); however, no significant changes in cross-sectional area occurred after 24 h (Fig. 4b). BSA-loaded implants did not undergo an initial loss in area, increasing to 1.74 ± 0.11-fold the initial cross-sectional area after 1 day, with no significant changes occurring throughout the remainder of the study. Implants that contained the combination of BSA/DiI initially shrink, returning to the initial area after 4 h. The implants then continued to swell through 4 days, and achieved a maximum area of 1.41 ± 0.06-fold the initial cross-sectional area, with no significant changes occurring through the remainder of the study.

Erosion and Bathside Uptake

Initially, all implant formulations had masses greater than the maximum theoretical polymer mass, indicating the presence of residual solvent (Fig. 5a). Implants without excipients and implants incorporating water as the additive had significantly lower masses than all other implant formulations after 7 days ($99.1 \pm 5.1\%$ and $94.5 \pm 1.8\%$ of the initial polymer mass, respectively), and were below the theoretical polymer mass, indicating the onset of erosion. Within 10 days, implants that incorporated water as the excipient had a significantly lower mass than all other implant formulations. Implants with DiI, BSA, and BSA/DiI still contained residual solvent on day 10 ($104.1 \pm 2.1\%$, $101.9 \pm 0.7\%$, and $104.8 \pm 0.8\%$ of the theoretical polymer mass, respectively), but by 14 days all implant formulations had begun eroding. No statistical differences were observed in the erosion profile of implants containing DiI, BSA, or BSA/DiI (Fig. 5a).

Significant differences in fluid uptake occurred between all groups after 3 days in solution and these differences were maintained until after 10 days in the bathside solution (Fig. 5b). The excipient-free control demonstrated the greatest fluid uptake, followed by implants containing water, then those with BSA, which was followed by those with BSA/DiI, and finally implants with DiI alone. Implants without an excipient had a 4.1 ± 0.1-fold increase in the initial implant mass after 7 days in PBS. The initial 7-day period of fluid uptake was followed by a decrease in the wet mass of the implants over the remainder of the study (Fig. 5b). The implant with water incorporated into the casting solution followed a similar pattern, but had a significantly lower fluid uptake than the excipient free control, reaching a maximum 3.1 ± 0.1-fold increase in implant mass after 7 days. Implants containing BSA rapidly reached a steady state, and were 2.4 ± 0.01-fold the initial implant mass after 24 h in the nonsolvent, with no significant changes in the wet mass of the implants occurring until after 14 days (Fig. 5b). After 14 days, a decrease in the wet mass was observed throughout the remainder of the study. Implants that contained a combination of BSA/DiI had a 1.9 ± 0.03-fold increase in mass after 1 day in the bathside solution, and no significant changes
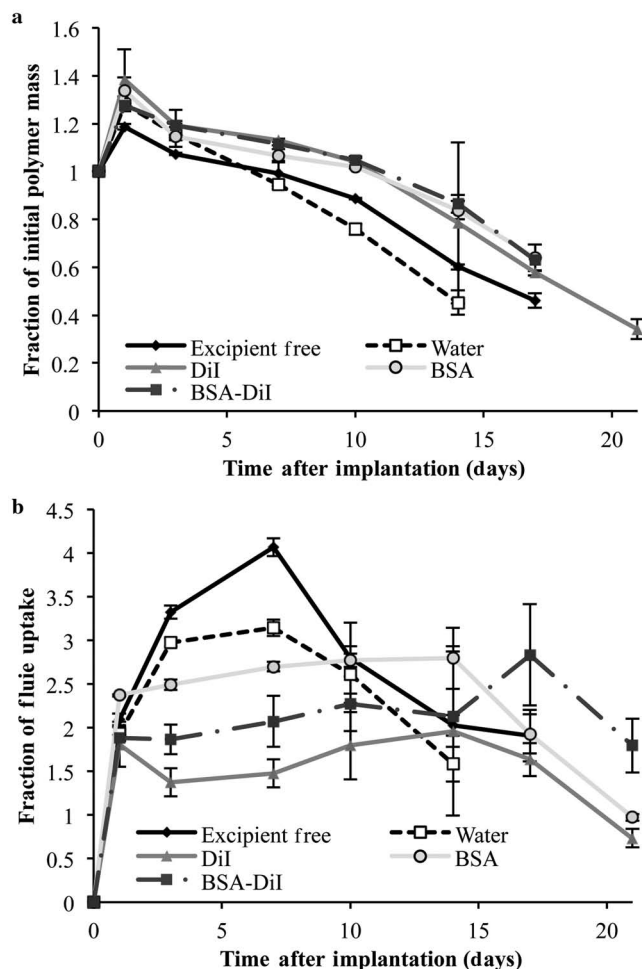
a

b

Figure 5.   Implant erosion over time (a) and the change in fluid uptake over time (b). All error bars represent standard deviation with $n = 3$.

occurred in the implant mass afterward. Implants that contained DiI had an initial period of fluid uptake resulting in a 1.8 ± 0.3-fold increase in the implant mass, with no significant changes observed until after 17 days in the bathside solution. On the final day of the study, a significant decrease in the implant mass was observed, resulting in an implant with a wet mass 0.7 ± 0.1-fold the initial mass of the implant.

Implant Microstructure Analysis

Within 3 days, the microstructure of implants without an excipient had a shell and an interior spongy domain, with a central region that still had residual solvent (Fig. 6). This interior spongy domain consisted of a small interconnected porous network, of polymer-rich and polymer-lean domains. The incorporation of water into the casting solution resulted in a decrease in the implant shell thickness, and resulted in the formation of a continuous porous network throughout the implant structure, including the shell. Incorporation of DiI into the polymer solution resulted in an implant with a thinner shell than the excipient-free control, but with larger less interconnected pores in the interior of the implant. Incorporation of BSA resulted in a thick outer shell similar to what is observed in the excipient-free control. The interior domain had smaller pores than what

was observed when DiI was included in the casting solution, but the pores were less interconnected than the excipient-free control. The combination of BSA/DiI resulted in an implant with a microstructure similar to what was observed with implants containing BSA alone. The implants containing BSA/DiI also had a few larger pores in the interior domain of the implants similar to what was observed with implants containing only DiI (Fig. 6).

After 7 days, the shell of the excipient-free control became thinner and the interior domain became more porous and interconnected (Fig. 7). The microstructure of implants that incorporated water into the casting solution had a less dense shell and interior domain, with greater interconnectivity of the porous interior domain. Implants containing DiI developed larger pores just beginning to become interconnected (Fig. 7). Although the shell remained dense, polymer-lean domains were also beginning to form. Implants that contained BSA did not significantly change between day 3 and day 7. The microstructure of implants with the combination of BSA/DiI appeared more dense, with a less interconnected porous network than the excipient-free control, and an increase in the size of the polymer-lean domains (Fig. 7).

DISCUSSION

Additives provide a means by which the drug release profile of ISFIs can be specifically tailored at unique phases of the release profile, without making changes to the polymer or the solvent, which can have adverse affects on the solution viscosity. The effect that the additive has on drug release is dependent on the way in which the excipient interacts with the surrounding matrix, payload, or how it alters the mass transfer kinetics of the solvent/nonsolvent.[5,8,35] For highly miscible solvents, the polymer precipitates instantaneously resulting in a dense polymer shell.[5] Within the implant interior, polymer-lean droplets consisting of a mixture of solvent and nonsolvent form. These droplets will aggregate into larger domains unless a sufficient concentration of nonsolvent is present, which stabilizes the structure by causing the polymer to precipitate.[5,6] This process results in the formation of a highly interconnected porous network, observed in the implants without excipients. The interconnectivity of the pores was hypothesized by McHugh and coworkers[9,21–23] to provide a network for drug diffusion ultimately leading to elevated burst release, which occurs as drug is depleted from these polymer-lean regions. As a consequence of the structure, diffusion is limited resulting in the characteristic release profile.

We hypothesized that the incorporation of DiI into the polymer solution would function to increase the hydrophobicity of the polymer solution and reduce water uptake. The increased hydrophobicity and decrease in water uptake resulted in delayed demixing to occur after the initial shell formation. As a consequence of the delayed demixing, the polymer-lean domains could not be stabilized by the nonsolvent and aggregated, resulting in the observed microstructure that consisted of large pores with little interconnectivity (Fig. 6). The limited interconnectivity of the pores reduced burst release and limited diffusion of drug through the implant.

Incorporation of nonsolvent into the casting solution has been used to control the microstructure of asymmetric and symmetric membranes fabricated using phase inversion.[1,5,10,11,18,35]
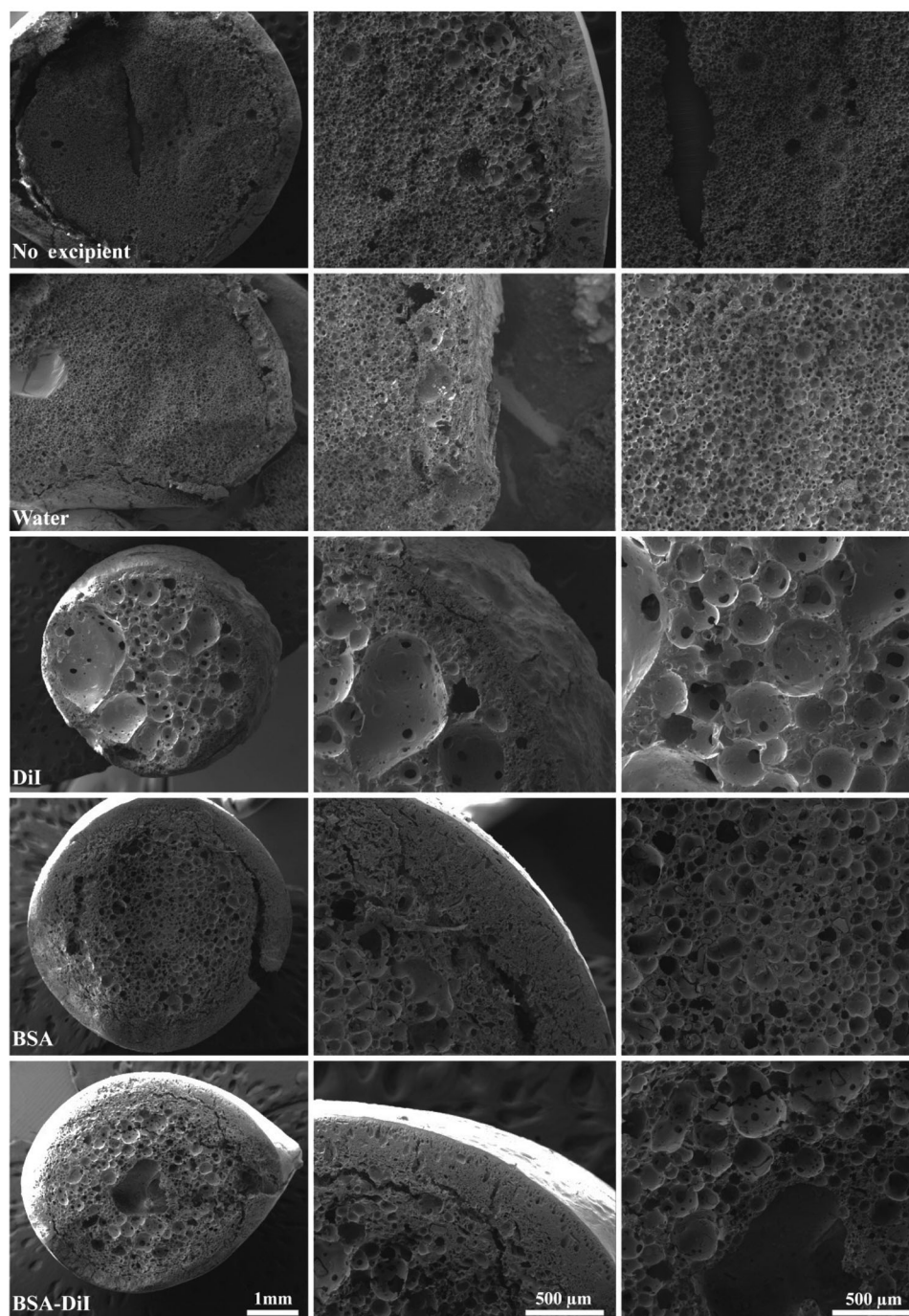
Figure 6. Scanning electron microscopy images of implants in the bath solution after 3 days. Each row is representative of implants loaded with different excipients. Column 1 is a low magnification image of the entire implant, column 2 is an image of the shell, and column 3 is an image of the center of the implant.

Therefore, water was added to the casting solution in order to reduce the solvent/nonsolvent gradient. As a consequence of the reduced solvent exchange, lower fluid uptake was observed, as well as a decrease in burst release that we hypothesize to be a result of a decrease in solvent transport. SEM analysis of the microstructure shows that incorporating water into the polymer solution also reduced the shell thickness of the implants, which we hypothesize resulted in the enhanced release of drug during the diffusion phase of release.

Through ultrasound imaging, all of the implants were observed to become hollow if given a large enough timescale (Figs. 1 and 3). A similar observation has been described in previous studies.[14,19,28,36] We hypothesize that the development of this macroscale feature occurs as a result of auto-catalytic degradation and can be explained with percolation theory.[19,37–39] Because of the removal of the acidic degradation biproducts from the surface of the implant by the nonsolvent, and diffusion limitations of the acidic biproducts imparted by
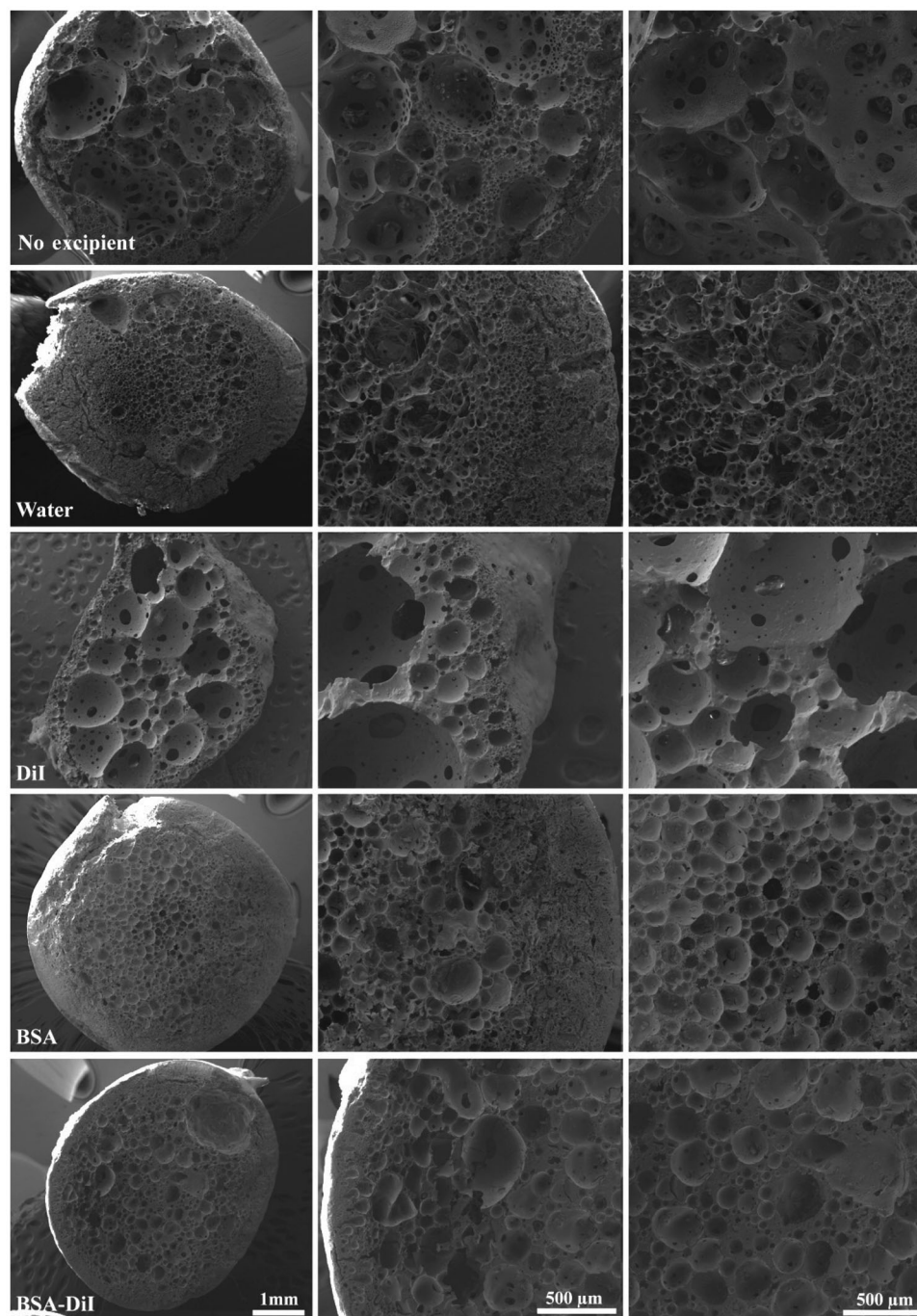
Figure 7. Scanning electron microscopy images of implants in the bath solution after 7 days. Each row is representative of implants loaded with different excipients. Column 1 is a low magnification image of the entire implant, column 2 is an image of the shell, and column 3 is an image of the center of the implant.

the shell, a pH gradient develops within the implant.[19,37,40–42] The buildup of acidic biproducts continues until the matrix that makes up the shell erodes below the percolation threshold, resulting in a continuous path through which the oligomers can diffuse out of the implant, and consequently causes the formation of a pore in the center of the implant.[19] Recent studies by Schädlich et al.[43] have demonstrated that the internal pH of an ISFI decreases below 3 within the first 6 days in the bathside solution, and is followed by an increase in pH, indicating loss of the acidic biproducts.

We also hypothesize that the increased diffusion of the implants that contained water in the casting solution reduced the buildup of degradation biproducts and resulted in the prolonged formation of the central pore relative to the excipient-free control. In previous studies, DiI was shown to enhance the degradation of ISFIs.[14] Therefore, we postulate that the reduced interconnectivity, dense polymer regions, and elevated hydrophobicity within the implants containing DiI result in a greater decrease in pH within the macropores enhancing the autocatalysis effect. Incorporation of BSA into ISFIs has been

shown to reduce polymer erosion and degradation, and was not anticipated to alter burst release.[14] BSA is not soluble in the polymer solution, and was suspended before injection into the bathside solution. The BSA suspension provided points of acoustic impedance mismatch within the polymer solution resulting in an initial elevated backscatter. This increase in echogenicity was lost within a half hour because of diffusion of water into the polymer solution. Interestingly, a decrease in signal was observed after 48 h, which we hypothesize to be a result of increased polymer density attenuating the ultrasound signal transmitted through the implant. SEM images showed that the implants had a denser, less interconnected porous microstructure than the excipient-free control, which we hypothesize functioned to reduce drug release during the diffusion period of release. Furthermore, because of the isoelectric point of the BSA, the pH gradient that develops in the implant would serve to facilitate polyionic complexation of the degraded oligomers that function to maintain the matrix architecture ultimately reducing polymer erosion and delaying the onset of degradation-facilitated release. The reduction in release may also be effected by interaction of fluorescein with BSA, which would also serve to reduce release during the diffusion and degradation phases of drug dissolution.

When a combination of DiI/BSA was incorporated into the casting solution, synergistic effects of both excipients were observed. A dense polymer shell was formed similar to what was observed with implants containing BSA alone (Fig. 6). Because of the reduced fluid uptake, a period of delayed demixing occurred, and similar to what was observed with implants containing DiI, the polymer-lean domains aggregated resulting in the observed increase in size and decrease in interconnectivity of the porous network between days 3 and 7 (Figs. 6 and 7). The subsequent changes in microstructure most likely resulted in a decrease in the dissolution of drug during the diffusion period of release.

CONCLUSIONS

Medical imaging systems provide a powerful tool by which the long-term *in situ* behavior of implants can be evaluated nondestructively and aid in the rational design of implant formulations. Excipients provide an ideal method by which the release profile can be tailored for specific applications. The findings in this study have demonstrated that excipients can be used to reduce burst release, alter diffusivity, as well as delay the onset of degradation-facilitated release. Understanding how the additives alter microstructure and interact with the matrix can be used to tailor the release profile of ISFIs for specific applications. It has been demonstrated that changes in the microstructure can significantly alter the release profile.[14,22,23,44] Currently, only a handful of additives have been evaluated,[30,31,45] with a large focus of research evaluating the role of solvents and the polymer.[21,24,25,29,46–51] These findings demonstrate that the use of additives can significantly alter the implant release profile, within the different periods of release.

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