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Effect of the Subcutaneous Environment on Phase Sensitive *In Situ* Forming Implant Drug Release, Degradation, and Microstructure

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Abstract

In situ forming implants are a promising platform used for the release of therapeutic agents. Significant changes in behavior occur when the implants are used *in vivo* relative to implants formed *in vitro*. To understand how the injection site effects implant behavior, poly(lactic-co-glycolic acid) implants were examined after injection in the subcutaneous space of a Sprague Dawley rat model to determine how the environment altered implant erosion, degradation, swelling, microstructure and mock drug release. Changes in implant microstructure occurred over time for implants formed *in vivo*, where it was observed that the porosity was lost over the course of 5 d. Implants formed *in vivo* had a significantly greater burst release ($p < 0.05$) relative to implants formed *in vitro*. However during the diffusion period of release, implants formed *in vitro* had a significantly higher daily release (2.1%/day, $p < 0.05$), which correlated to changes in implant microstructure. Additionally, implants formed *in vitro* had a 2 fold increase in the first order degradation kinetics relative to the implants formed *in vivo*. These findings suggest that the changes in implant behavior occur as a result of changes in the implant microstructure induced by the external environment.

Keywords

phase inversion; *in situ* forming implant; PLGA; implant microstructure; drug release

1. Introduction

Phase sensitive *in situ* forming implants (ISFI) have been successfully used to deliver a variety of therapeutic agents¹⁻¹⁰ including antibiotics for the treatment of periodontitis,

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insulin to assist in maintaining basal levels of the protein, as well as leuprolide acetate to treat late-stage prostate cancer¹¹⁻¹⁷. The implants, first developed by Dunn *et al.*, are a liquid solution outside of the body but transition into solid drug eluting depots upon contact with an aqueous environment through a process known as phase inversion^{18,19}. Phase inverting ISFIs are typically manufactured by dissolving a hydrolytically degradable bioresorbable polymer with a biocompatible solvent and creating a suspension or solution with the therapeutic agent. Phase inversion occurs as a result of counter transport between solvent and nonsolvent, resulting in the precipitation of the polymer from solution^{7,8,10}.

Any factor that can alter the mass transfer kinetics of ISFIs can be used to alter the release profile. Factors such as polymer molecular weight (Mw), solvent, drug/polymer interactions, or the stiffness of the injection site have been shown to alter the release behavior of these implants^{3,4,7-10,20-26}. Furthermore, the implant microstructure that forms as a result of the rate of polymer precipitation has also been shown to drastically alter the release of drug by changing the interconnectivity of the porous structures within the implant^{7-9,22,23}. While the *in vivo* environment has been shown to change implant behavior, little has been done to determine the mechanisms that drive *in vitro/in vivo* disparities. The ability to correlate drug release data between *in vitro* and *in vivo* systems, known as the *in vitro-in vivo* correlation (IVIC) provides a tool that can be used to minimize both the time and cost involved in drug development by reducing the number of human studies required during formulation and development^{27,28}. However establishment of accurate IVIVC for biodegradable systems has been challenging due to the complexity of these release systems²⁸. Therefore, by determining factors that lead to poor IVIVC more accurate release systems can be developed to more accurately predict how drug eluting systems will behave *in situ*.

These *in vivo* effects are particularly pronounced with small molecular weight hydrophilic drugs²⁵, limiting the use of these implants with newer more effective therapies such as targeted kinase inhibitors used to treat cancer. Therefore, elucidating how the *in vivo* environment alters the release profile is paramount for improving the safety and utility of ISFIs. In order to determine the effects of the injection site on drug release, while minimizing the matrix/drug interactions, the small molecular weight compound sodium fluorescein was used. This molecule has been shown to have a similar release profile to the chemotherapeutic agent Doxorubicin within the first 14 d of release for phase sensitive ISFIs, without the associated toxicity⁹. The effect of the implant injection site on polymer erosion, degradation and microstructure were also evaluated.

Changes in mock drug sodium fluorescein release were evaluated through standard dissolution studies and analysis of implant contents post mortem. Implant erosion and fluid uptake were measured through changes in the implant mass. Changes in polymer degradation were evaluated using gel permeation chromatography (GPC), and implant microstructure was evaluated using scanning electron microscopy (SEM). Results from this study should provide insight into the factors that alter the release profile of implants formed in the subcutaneous space and those formed *in vitro*.

2. Materials and Methods

2.1. Materials

Poly(DL-lactic-*co*-glycolic acid) (acid-capped PLGA, 50:50, Mw 21,000 Da) was obtained from Evonik Industries (Essen, Germany) and used as received. N-methyl-2-pyrrolidinone (NMP) and sodium fluorescein were obtained from Sigma Aldrich (St. Louis, MO) and used as received.

2.2. Polymer Solution Formulation

Polymer solutions were prepared using a 39:60:1 mass ratio of PLGA:NMP:fluorescein. Fluorescein was dissolved in NMP for 30 m before addition of PLGA as previously described^{9,26} and stirred overnight in a 37°C shaker table at 90 RPM. Polymer solutions were used within three days.

2.3. Mock Drug Release

In Vitro Release—Mock drug release profiles were evaluated as previously described^{9,26}. Polymer solution (40.9±3.9 mg) was injected into 10 ml of 37°C PBS (pH 7.4), and maintained at 37°C in an incubated orbital shaker at 90 RPM. The bath side solution was sampled and then replaced with fresh PBS after 1 and 4 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 every other day for 1 week. After 7 d, implants were removed from the bath solution and degraded in 5 ml of 2M NaOH. Fluorescein mass was determined by comparing the sampled solution with a standard curve of known fluorescein concentrations with a multimode microplate reader (Tecan Ltd., Infinite 200 series) at excitation/emission wavelengths (Ex/Em) of 485/525 nm. The cumulative mock drug release was calculated from these measurements and normalized by the total mass of mock drug in the implant.

In Vivo Release—All animal studies were performed as previously described following protocols approved by the Case Western Reserve University Institutional Animal Care and Use Committee^{25,29}. Briefly, 18 12-week old male Sprague-Dawley rats with an average body weight of 303±18 g (Charles River Laboratories Inc., Wilmington, MA) were anesthetized using 1% isoflurane with an oxygen flow rate of 1 l/min (EZ150 Isoflurane Vaporizer, EZ AnesthesiasTM). Implant solution (69.6±9.4 mg) was injected under the dorsal skin flap in four locations using a 21-gauge hypodermic needle. Mock drug release was determined by dissecting out the implants after euthanasia, then degrading the implants in 5 ml of 2 M NaOH overnight. The mass of fluorescein was determined by comparison to a standard curve using a Tecan 200 series plate reader at an excitation wavelength of 485 nm and an emission wavelength of 525 nm^{25,29}.

2.4. Erosion, Degradation, and Bathside Uptake

Changes in implant mass over time were monitored as describe previously⁹. First, the initial mass of implants added to 10 ml of warm PBS (pH 7.4) was recorded and maintained at 37°C in an incubated shaker rotating at 90 RPM. Implants were removed from the bath solution at predefined time points (1 h, 4 h, 1 d, 3 d, 5 d, and 7 d). At each time point, implants were dried with a clean paper towel, and then weighed in order to obtain the wet

mass. Implants dissected from the subcutaneous space were cleaned and the wet mass was recorded. All implants were then frozen, lyophilized for 4 days, and weighed when completely dry. 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.

Changes in the Mw were determined through GPC analysis as previously described^{9,26}. Briefly, after lyophilization, implants were dissolved in tetrahydrofuran (THF) and the solution was then filtered using a 0.45µm syringe filter. The Mw was determined relative to narrow polystyrene standards using an Agilent 1200 series liquid chromatography system, a refractive index and variable wavelength detector, and two American Polymer Standards linear bed GPC columns (Mentor, OH) at a flow rate of 1 ml/min.

2.5. Scanning Electron Microscope Imaging and Analysis

Implant microstructure was evaluated as previously described^{9,26}. Briefly, the implants were freeze fractured and then lyophilized for 4 d. Next, the implants were sputter coated with 5 nm of palladium and imaged using a Quanta 200 3D ESEM with an acceleration voltage of 3.5 kV and a hole size of 10.

2.6. Statistical Analysis

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

3. Results

3.1. Mock Drug Release

The mock drug dissolution kinetics of ISFIs include a period of burst (24 h), followed by diffusion (>24 h), and culminating in degradation facilitated release. Significantly more mock drug was released from implants formed in the subcutaneous space than those formed *in vitro* (Figure 1). The cumulative release over the 7 d study was $86.9 \pm 5.2\%$ and $49.5 \pm 3.6\%$ for implants formed *in vivo* and *in vitro* respectively. In addition a significantly higher burst release was seen in *in vivo* samples relative to implants formed *in vitro* ($66.7 \pm 13.4\%$ compared with $30.1 \pm 6.1\%$, $p = 0.05$). During the diffusion phase of release, no significant mock drug release occurred with samples formed *in vivo*. Conversely, diffusion facilitated release occurred at a rate of 2.1% of mock drug per day for implants formed *in vitro* (Figure 1).

3.2. Erosion, Degradation, and Bathside Uptake

A rapid initial loss of solvent was seen in all implants. The bulk solvent loss occurred within the first 24 h, with the rate becoming more gradual throughout the duration of the study. Implants formed *in vitro* contained residual solvent throughout the 7 d study ($103.5 \pm 0.3\%$). Implants formed *in vivo* required 5 days in order to release all of the residual solvent, with

implant mass being $94.2 \pm 11.6\%$ of the total polymer mass after 7 d. No statistical differences were observed between the rates of polymer erosion with *in vitro* and *in vivo* studies (Figure 2A).

Degradation occurred at a significantly faster rate *in vitro* compared with *in vivo* degradation, with the first order degradation rate constant 2 times greater for *in vitro* samples. Implants formed *in vitro* degraded to $20.9 \pm 0.4\%$ of the initial Mw after 7 d in PBS compared with implants formed *in vivo* which degraded to $38.9 \pm 3.8\%$ initial mass in that same time period. Statistically significant differences in polymer Mw were observed after 24 h, between implants formed *in vitro* composed of those formed *in vivo* ($P=0.017$). Statistical differences in Mw were observed throughout the remainder of the study (Figure 2B).

Implants formed *in vitro* showed a rapid initial period of fluid uptake, followed by continued uptake through the duration of the study, reaching a maximum mass 3.9 ± 0.1 fold more than the initial implant. Fluid uptake for implants formed in the subcutaneous space reached a maximum 4 h after implantation (2.4 ± 0.4 fold), followed by a loss of mass through 5 d reaching a minimum of 1.2 ± 0.3 fold. No statistical differences were observed in fluid uptake until after 24 h, at which time statistical differences were observed through the duration of the study, with *in vitro* samples absorbing more fluid than implants formed *in vivo* (Figure 2C).

3.3. Implant Microstructure Analysis

Distinct differences in implant microstructure were observed between implants formed *in vivo* relative to those formed *in vitro* (Figure 3). Cryosectioning of the implants formed *in vitro* could not be performed until 3 d after formation due to the presence of residual solvent. The microstructure of these implants consisted of a central pore caused by the residual solvent. This central domain was surrounded by a porous cell-like microstructure with a high level of interconnectivity, a high surface area, and a thick outer shell (Figure 4). Over time the residual solvent was lost and the pores of the spongy domain became larger while the shell thickness decreased (Figure 4). Implants formed *in vivo* lost solvent more rapidly and could be cryosectioned as soon as 4 h after injection into the subcutaneous space. After 4 h, these implants had a similar porous cell like microstructure as implants formed *in vitro*. This structure was lost within 72 h, resulting in a decrease in surface area relative to the *in vitro* samples. No discernable outer shell was observed after 120 h with a continued loss of porous structure occurring throughout the study (Figure 5).

4. Discussion

ISFIs are a promising platform for the delivery of a number of agents, ranging from antibiotics to chemotherapeutics^{12,13,30,31}. The minimally invasive delivery of these depots provides a unique system that can be used to achieve elevated local concentrations of drugs without systemic involvement³². While a promising platform, ISFIs have not found widespread use in the clinic. The dearth of clinical applications can partly be explained by the sensitivity of implant behavior to a number of factors including the polymer type, solvent, and drug properties^{6,7,9,14,20-22}. Furthermore, the effect of the injection site

properties can also play a role in altering implant behavior²⁵. The purpose of the study was to determine how the *in vivo* environment alters the implant behavior.

Significant differences were observed in the microstructures for implants formed *in vivo* and *in vitro*. The implants formed *in vivo* showed a gradual loss of porosity, while the porosity increased for implants formed *in vitro* (Figure 5). This transition in microstructure was a significant observation because such drastic changes in implant morphology are not observed *in vitro*, suggesting that the environment alters implant behavior beyond what has currently been hypothesized. Our findings show that the initial microstructure of implants formed in the subcutaneous space is reminiscent of what one would expect from a fast phase inverting system, with a highly interconnected porous network that would facilitate rapid drug release. However, over time the microstructure unexpectedly transitions into a dense matrix of polymer that would ultimately function to reduce drug diffusion from the implant. The final microstructure is similar to what is observed when using extremely hydrophobic solvents such as ethyl benzoate to form an implant, and the resultant microstructure occurs as a result of limited solvent/non-solvent (such as water) exchange, limiting the formation of polymer lean domains within the implant interior, thus forming a more polymer rich implant than what is observed in the highly porous fast phase inverting systems.

We hypothesize that these changes occur as a result of increased pressure on the implants by the injection site²⁵. Initially there is a period of rapid solvent loss and fluid uptake, similar to what is observed with implants formed *in vitro* (Figure 2). However, over the course of 72 h, the absorbed fluid is expelled from the implants, which we hypothesize to be the result of compressive forces from the surrounding tissue acting on the implants²⁵. The loss of the stabilizing nonsolvent and the presence of residual solvent facilitates the entropically driven aggregation of the hydrophilic polymer lean domains into larger poorly interconnected structures observed within the interior of the implants (Figure 5). Over time, under continued mechanical compression by the surrounding tissue, fluid would continue to be expelled, resulting in the continued loss of polymer lean domains that consequently leads to the loss of porosity within the implant.

Consistent with previous studies, mock drug release was greater for implants formed *in vivo* than those formed *in vitro* (Figure 1)^{9,25,26,29}. We hypothesize that the significant increase in burst release occurs as a result of suppressed implant expansion by the surrounding tissue, leading to a reactionary force from the tissue causing the expulsion of fluid from the implant and subsequently increasing burst release. Furthermore, changes in surface-to-volume ratio and the bathside composition of the subcutaneous space may also contribute to the increase in burst release by implants *in vivo*. Interestingly, after the initial period of elevated burst release *in vivo*, only a limited mass of mock drug was released during the diffusion phase of dissolution. We hypothesize that the loss of diffusivity of the implants occurs as a result of the changes in implant porosity, which ultimately reduced the rate of release during the diffusion period of drug dissolution^{8,22,33}.

No statistical differences were observed with implant mass loss during the course of this study between implants formed *in vivo* and *in vitro*. These findings are consistent with previous studies where no significant loss of polymer occurs in less than 10 d^{9,26}. While we

anticipated that the *in vivo* environment would result in elevated degradation³⁴, we hypothesize that the differences in the degradation rates between *in vitro* and *in vivo* implants are caused by both the higher water content as well as the higher surface area of the implants formed *in vitro*. Additionally, the differences in polymer degradation could alter the implant osmolality and be a factor in altering the fluid uptake for the two conditions.

It has been demonstrated that implant formulations that alter the microstructure can significantly change the release profile^{7-9,22}. Currently only a handful of studies have evaluated the effect of the host on implant behavior. These findings demonstrate that the host environment significantly alters the implant microstructure, which not only changes the mock drug release profile, but the rate of degradation, and fluid uptake as well. Systems such as apparatus 4 have been designed to include laminar flow, turbulent flow, multiple compartments, as well as changes in pH to improve IVIVC³⁵. Therefore development of release systems that can more accurately mimic the effects of injection site may lead significant improvements in the IVIVC of ISFIs and improve the transition of a therapeutic systems into the clinical practice.

5. Conclusions

It has been hypothesized that local compressive mechanical forces can alter the drug release profile of ISFIs. While studies have evaluated the effect of injection site on implant behavior, few studies have investigated the underlying changes in implant structure that elicit these changes. Our findings suggest that changes in implant microstructure that occur *in situ*, can significantly alter the drug release and degradation rate of the implants. Therefore, by designing implants that will reduce these changes in microstructure, the correlation between *in vitro/in vivo* drug release profiles can be achieved.

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References

1. Al-Tahami K, Meyer A, Singh J. Poly lactic acid based injectable delivery systems for controlled release of a model protein, lysozyme. *Pharmaceutical Development and Technology*. 2006; 11(1): 79–86. [PubMed: 16544911]
2. Al-Tahami K, Oak M, Singh J. Controlled delivery of basal insulin from phase-sensitive polymeric systems after subcutaneous administration: In vitro release, stability, biocompatibility, in vivo absorption, and bioactivity of insulin. *J Pharm Sci*. 2011; 100(6):2161–2171. [PubMed: 21491440]
3. Astaneh R, Erfan M, Barzin J, Mobedi H, Moghimi H. Effects of Ethyl Benzoate on Performance, Morphology, and Erosion of PLGA Implants Formed In Situ. *Advances in Polymer Technology*. 2008; 27(1):17–26.
4. Astaneh R, Erfan M, Mobedi H, Moghimi HR. Formulation of an Injectable Implant for Peptide Delivery and Studying the Effect of Polymer Molecular Weight on Its Release Behavior. *Journal of Peptide Science*. 2004; 10:142–142.

5. Astaneh R, Nafissi-Varcheh N, Erfan M. Zinc-leuprolide complex: preparation, physicochemical characterization and release behaviour from in situ forming implant. *Journal of Peptide Science*. 2007; 13(10):649–654. [PubMed: 17726721]
6. Berges R, Bello U. Effect of a new leuporelin formulation on testosterone levels in patients with advanced prostate cancer. *Curr Med Res Opin*. 2006; 22(4):649–655. [PubMed: 16684425]
7. Brodbeck KJ, DesNoyer JR, McHugh AJ. Phase inversion dynamics of PLGA solutions related to drug delivery - Part II. The role of solution thermodynamics and bath-side mass transfer. *Journal of Controlled Release*. 1999; 62(3):333–344. [PubMed: 10528071]
8. Graham PD, Brodbeck KJ, McHugh AJ. Phase inversion dynamics of PLGA solutions related to drug delivery. *Journal of Controlled Release*. 1999; 58(2):233–245. [PubMed: 10053196]
9. Solorio L, Olear AM, Zhou H, Beiswenger AC, Exner AA. Effect of cargo properties on in situ forming implant behavior determined by noninvasive ultrasound imaging. *Drug Deliv Transl Res*. 2012; 2(1):45–55. [PubMed: 22712054]
10. Solorio, L.; Solorio, LD.; Gleeson, S.; Olear, AM.; Carlson, AC.; Exner, AA. Phase Inverting Polymer Systems In Drug Delivery And Medicine. In: Ehlers, TP.; Wilhelm, JK., editors. *Polymer Phase Behavior*. Nova Science Publishers, Inc; 2011.
11. Dhawan S, Kapil R, Kapoor DN. Development and Evaluation of In Situ Gel-forming System for Sustained Delivery of Insulin. *J Biomater Appl*. 2011; 25(7):699–720. [PubMed: 20207780]
12. Dunn RL, Garrett S. The drug delivery and biomaterial attributes of the ATRIGEL technology in the treatment of periodontal disease. *Expert opinion on investigational drugs*. 1998; 7(9):1483–1491. [PubMed: 15992045]
13. Gad HA, El-Nabarawi MA, El-Hady SSA. Formulation and Evaluation of PLA and PLGA In Situ Implants Containing Secnidazole and/or Doxycycline for Treatment of Periodontitis. *Aaps Pharmscitech*. 2008; 9(3):878–884. [PubMed: 18654864]
14. Kang FR, Singh J. In vitro release of insulin and biocompatibility of in situ forming gel systems. *International Journal of Pharmaceutics*. 2005; 304(1-2):83–90. [PubMed: 16181752]
15. Krupka TM, Weinberg BD, Ziats NP, Haaga JR, Exner AA. Injectable polymer depot combined with radiofrequency ablation for treatment of experimental carcinoma in rat. *Investigative Radiology*. 2006; 41(12):890–897. [PubMed: 17099428]
16. Ravivarapu HB, Moyer KL, Dunn RL. Sustained activity and release of leuprolide acetate from an in situ forming polymeric implant. *Aaps Pharmscitech*. 2000; 1(1):E1. [PubMed: 14727850]
17. Ravivarapu HB, Moyer KL, Dunn RL. Sustained suppression of pituitary-gonadal axis with an injectable, in situ forming implant of leuprolide acetate. *J Pharm Sci*. 2000; 89(6):732–741. [PubMed: 10824131]
18. Dunn, RL.; English, JP.; Cowsar, DR.; Vanderbilt, DP. Biodegradable *in situ* forming implants and methods of producing the same. Patent US. 5990194A. 1999.
19. Dunn, RL.; Tipton, AJ. Polymeric compositions useful as controlled release implants. Patent US. 5702716A. 1997.
20. Astaneh R, Erfan M, Moghimi H, Mobedi H. Changes in morphology of in situ forming PLGA implant prepared by different polymer molecular weight and its effect on release behavior. *J Pharm Sci*. 2009; 98(1):135–145. [PubMed: 18493999]
21. Bakhshi R, Vasheghani-Farahani E, Mobedi H, Jamshidi A, Khakpour M. The effect of additives on naltrexone hydrochloride release and solvent removal rate from an injectable in situ forming PLGA implant. *Polymers for Advanced Technologies*. 2006; 17(5):354–359.
22. DesNoyer JR, McHugh AJ. Role of crystallization in the phase inversion dynamics and protein release kinetics of injectable drug delivery systems. *J Control Release*. 2001; 70(3):285–294. [PubMed: 11182199]
23. DesNoyer JR, McHugh AJ. The effect of Pluronic on the protein release kinetics of an injectable drug delivery system. *J Control Release*. 2003; 86(1):15–24. [PubMed: 12490369]
24. Patel RB, Carlson AN, Solorio L, Exner AA. Characterization of formulation parameters affecting low molecular weight drug release from in situ forming drug delivery systems. *Journal of Biomedical Materials Research Part A*. 2010; 94A(2):476–484. [PubMed: 20186771]

25. Patel RB, Solorio L, Wu HP, Krupka T, Exner AA. Effect of injection site on in situ implant formation and drug release in vivo. *Journal of Controlled Release*. 2010; 147(3):350–358. [PubMed: 20728486]
26. Solorio L, Olear AM, Hamilton JI, Patel RB, Beiswenger AC, Wallace JE, Zhou H, Exner AA. Noninvasive characterization of the effect of varying PLGA molecular weight blends on in situ forming implant behavior using ultrasound imaging. *Theranostics*. 2012; 2(11):1064–1077. [PubMed: 23227123]
27. Cardot JM, Davit BM. In vitro-in vivo correlations: tricks and traps. *AAPS J*. 2012; 14(3):491–499. [PubMed: 22547350]
28. Emami J. In vitro - in vivo correlation: from theory to applications. *J Pharm Pharm Sci*. 2006; 9(2): 169–189. [PubMed: 16959187]
29. Solorio L, Babin BM, Patel RB, Mach J, Azar N, Exner AA. Noninvasive characterization of in situ forming implants using diagnostic ultrasound. *Journal of Controlled Release*. 2010; 143(2): 183–190. [PubMed: 20060859]
30. Sartor O. Eligard: leuprolide acetate in a novel sustained-release delivery system. *Urology*. 2003; 61(2 Suppl 1):25–31. [PubMed: 12667884]
31. Sartor O. Eligard (R) 6: A new form of treatment for prostate cancer. *European Urology Supplements*. 2006; 5(18):905–910.
32. Exner AA, Saidel GM. Drug-eluting polymer implants in cancer therapy. *Expert Opin Drug Deliv*. 2008; 5(7):775–788. [PubMed: 18590462]
33. Brodbeck KJ, Pushpala S, McHugh AJ. Sustained release of human growth hormone from PLGA solution depots. *Pharmaceutical Research*. 1999; 16(12):1825–1829. [PubMed: 10644069]
34. Lu L, Peter SJ, Lyman MD, Lai HL, Leite SM, Tamada JA, Uyama S, Vacanti JP, Langer R, Mikos AG. In vitro and in vivo degradation of porous poly(DL-lactic-co-glycolic acid) foams. *Biomaterials*. 2000; 21(18):1837–1845. [PubMed: 10919687]
35. Fotaki N, Aivaliotis A, Butler J, Dressman J, Fischbach M, Hempenstall J, Klein S, Reppas C. A comparative study of different release apparatus in generating in vitro-in vivo correlations for extended release formulations. *Eur J Pharm Biopharm*. 2009; 73(1):115–120. [PubMed: 19442728]

Figure 1.

Figure 2.

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