Published in final edited form as:

Int J Pharm. 2007 August 1; 340(1-2): 6–12.

BIOERODIBLE DEVICES FOR INTERMITTENT RELEASE OF SIMVASTATIN ACID

Ju Hyeong Jeon¹, Mark V. Thomas², and David A. Puleo^{1,*}

¹ Center for Biomedical Engineering, University of Kentucky, Lexington, KY, USA

² College of Dentistry, University of Kentucky, Lexington, KY, USA

Abstract

The association polymer system of cellulose acetate phthalate (CAP) and Pluronic F-127 (PF-127) was used to create intermittent release devices for mimicking the daily injection of simvastatin that has been reported to stimulate bone formation. To enhance solubility in water, prodrug simvastatin was modified by lactone ring opening, which converts the molecule to its hydroxyacid form. CAP/PF-127 microspheres incorporating simvastatin acid were prepared by a water-acetone-oil-water (W/A/O/W) triple emulsion process. Devices were then fabricated by pressure-sintering UV-treated blank and drug-loaded microspheres. Using a multilayered fabrication approach, pulsatile release profiles were obtained. Delivery was varied by changing loading, number of layers, blend ratio, and incubation conditions. To determine the cellular effects of intermittent exposure to simvastatin acid, MC3T3-E1 cells were cultured with either alternating or sustained concentrations of simvastatin acid in the medium, and DNA content, alkaline phosphatase activity, and osteocalcin secretion were measured. For all three cell responses, cultures exposed to simvastatin acid showed higher activity than did control cultures. Furthermore, cell activity was greater for cells cultured with intermittent concentrations of simvastatin acid compared to cells that were constantly treated. These results imply that devices intermittently releasing simvastatin acid warrant further study for locally promoting osteogenesis.

Keywords

Intermittent release; Controlled release; Pulsatile release; CAP/PF-127 system; Simvastatin acid

INTRODUCTION

The need to treat bone defects resulting from degenerative diseases, trauma, and reconstructive surgery continues to increase at a significant rate. More than 500,000 bone grafting procedures are performed annually in the United States (Popovic, 2001). Harvesting autogenous tissues requires an additional surgery at the donor site that can result in its own complications, such as inflammation, infection, and chronic pain. Also, the total amount of bone that can be harvested is limited and creates a supply problem. Although allograft tissue is treated by freezing, freeze-drying, gamma irradiation, electron beam radiation, or ethylene oxide, because it is obtained from a donor, a risk of disease transmission from donor to recipient exists (Boyce

Address correspondence to: David Puleo, Ph.D. Wenner-Gren Lab Center for Biomedical Engineering University of Kentucky Lexington, KY 40506-0070 USA Tel: +1-859-257-2405 Fax: +1-859-257-1856 E-mail: puleo@uky.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

et al., 1999). These problems have led to development of drug delivery devices, synthetic materials, and tissue engineered constructs for use as alternatives to autografts and allografts in bone repair.

Simvastatin is a well-known member of the statin family. Statins are potent pro-drugs of hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors that block conversion of HMG-CoA to mevalonic acid, which is needed for cholesterol biosynthesis (Sugiyama et al., 2000). Simvastatin occupies a portion of the binding site for HMG-CoA, thus blocking access of substrate to the active site (Istvan and Deisenhofer, 2001). Mevalonic acid is a precursor not only of cholesterol but also of isoprenoids, such as geranyl pyrophosphate, which is important in the control of osteoclast-mediated bone resorption (Casey and Seabra, 1996). Statins offer additional benefits, such as promotion of new blood vessel growth (Kureishi et al., 2000) and anti-inflammatory effects (Davignon and Laaksonen, 1999). Most relevant to the present work, Mundy's group originally demonstrated that statins induce expression of bone morphogenetic protein 2 (BMP-2) and that they stimulate bone formation on the calvaria of mice following daily subcutaneous injections (Mundy et al., 1999). Subsequent studies have shown that oral dosing with simvastatin increases cancellous bone volume in rats (Maeda et al., 2001), and it also increases transverse area of fracture callus as well as mechanical properties compared to controls (Skoglund et al., 2002).

For local delivery of simvastatin, typical controlled release devices that attempt to achieve zero-order kinetics may not be the most effective. To mimic alternating concentrations resulting from daily injection or oral dosing, devices providing intermittent release profiles would be useful. We previously investigated an association polymer system based on blends of cellulose acetate phthalate (CAP) and Pluronic F127 (PF-127) for providing pulsatile release of macromolecules (Raiche and Puleo, 2003).

The objectives of this study were to develop devices that intermittently release simvastatin acid and demonstrate that exposure of osteoblastic cells to alternating concentrations of the drug enhances bioactivity.

MATERIALS AND METHODS

Hydrolysis of simvastatin

To decrease hydrophobicity of simvastatin (Aldrich, Milwaukee, WI), 42 mg were dissolved in 1 ml of 95% ethanol, and 1.5 ml of 0.1 M NaOH was added. The solution was heated at 50°C for 2 hours. Then the final solution was neutralized to pH 7.2 with 0.1 M HCl, and the volume was brought to 10 ml with deionized water (Kaesemeyer et al., 1999). Simvastatin acid (10 mM) was stored frozen at -20°C.

CAP/PF-127 Microspheres

Microspheres containing different weight fractions of CAP (Fluka, Buchs, Switzerland) and PF-127 (Sigma, St Louis, MO) were prepared by a water-acetone-oil-water (W/A/O/W) triple emulsion process. Two g of different polymer blends of CAP and PF-127 (7:3, 6:4 and 5:5 by weight of CAP:PF-127) were dissolved in acetone. One ml of phosphate-buffered saline (PBS), pH 7.4, with or without 100 µM simvastatin acid was added to the acetone solution and mixed into rapidly stirring corn oil and sonicated. The CAP/PF-127 suspension and 5% Triton X-100 were added to deionized water and then stirred to harden the microspheres for 5 minutes. Following collection, microspheres were washed three times with deionized water, filtered, and dried in vacuum up to seven days.

Release devices were made using a pressure-sintering process. Microspheres were first treated by UV radiation in a laminar flow hood for 30 minutes. Ten to 15 mg of UV-treated

microspheres containing simvastatin acid were placed in the wells of a Delrin mold (diameter, 6.2 mm; depth, 20 mm) and consolidated by applying 20 MPa pressure for 5 sec. Next, blank microspheres were added on top of the first layer, and pressure was reapplied. By repeating this process, six-, eight-, and ten-layer devices were prepared. To provide directional control of drug release, the bottom and sides of the devices were coated three times with 10% poly (lactic-co-glycolic acid) (75:25, $M_w \sim 75$ kDa; Alkermes, Cincinnati, OH) solution in methylene chloride.

In Vitro Release

Samples were immersed in 5 ml of 150 mM PBS, pH 7.4, and incubated at 37°C under either static or dynamic (shaking at 80 rpm) conditions using a MaxQ Mini 4450 shaker (Barnstead/Lab-Line, Dubuque, IA). Supernatant was collected and replaced daily to maintain a constant volume. To allow quantification of released simvastatin acid, CAP was precipitated from the 1 ml of supernatant samples by treatment with 0.1 ml of 0.1 M hydrochloric acid. Acid-treated supernatants were then centrifuged at 1000 rpm for 5 minutes. Solutions were placed into 96-well assay plates, along with simvastatin acid standards made by serial diluting from a 1 mM stock solution. The concentration of simvastatin acid was determined fluorometrically using a SpectraMAX Gemini XS ($\lambda_{ex}=390$ nm, $\lambda_{em}=413$ nm).

Profiles were predicted using the approach of Lee (1984), who proposed the use of nonuniform initial concentration distributions as a means to regulate the release of biomolecules from drug delivery devices. For erosion-controlled systems, Eq. 1 describes the fractional release from a planar sheet with initial biomolecule distribution $f(x)$, half-thickness a , and erosion rate constant B .

$$\frac{M}{M_{\infty}} = \frac{\int_{a-Bt}^a f(x) dx}{\int_0^a f(x) dx} \quad \text{Eq. 1}$$

Cell culture

MC3T3-E1 preosteoblastic cells (CRL-2593; ATCC, Manassas, VA) were seeded at a density of 15,000/cm² into 24-well tissue culture plates in α -Minimum Essential Medium (MEM) containing 10% fetal bovine serum (GIBCO/Invitrogen, Carlsbad, CA), 50 μ g/ml ascorbic acid (Sigma), 5 mM β -glycerophosphate (Sigma), and 0–1 μ M simvastatin acid. In a deviation from previous simvastatin treatment studies in the literature, medium was exchanged every day. In sustained release cultures, cells were constantly exposed to simvastatin acid at a fixed concentration, *i.e.*, the medium always contained the same concentration of drug. In intermittent release cultures, cells were exposed to alternating concentrations, *i.e.*, the medium varied between simvastatin-containing and drug-free medium. Multiple dilutions of simvastatin acid were selected based on preliminary cytotoxicity studies. Medium in control cultures was changed on the same schedule, but it did not contain simvastatin acid at any time.

Assays

After 3, 7, 10, and 14 days of culture, cells were rinsed twice with PBS and then lysed by sonication in a high salt solution (0.05M NaH₂PO₄, 2M NaCl, and 2mM EDTA). DNA standards were prepared by serial diluting calf thymus DNA in the high salt solution. Hoechst 33258 (final concentration, 0.5 μ g/ml; Sigma) was added to DNA standards and samples and allowed to react in the dark for 10 minutes (Raiche and Puleo, 2003; Labarca and Paigen, 1980). The amount of DNA in the samples was determined by measuring fluorescence ($\lambda_{ex}=356$ nm, $\lambda_{em}=458$ nm).

To measure alkaline phosphatase (AP) activity, cell lysate was incubated with substrate solution prepared by dissolving 10 mM of p-nitrophenyl phosphate (Sigma) in 0.6 M 2-amino-2-methyl-1-propanol buffer, pH 10 (Thompson and Puleo, 1995; Raiche and Puleo, 2004). After 30 minutes, 0.25 N NaOH was added to each well to immediately stop enzyme activity. Absorbance at 410 nm was measured with an MR5000 microplate reader (Dynatech Laboratories, Chantilly, VA), and the amount of substrate cleaved was determined using $\epsilon=1.7 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. Activity was expressed as nmol of substrate cleaved per minute and then normalized by DNA content.

For measuring osteocalcin (OCN) secretion, conditioned medium was assayed using an EIA kit (Biomedical Technologies, Stoughton, MA) following the manufacturer's protocol. Concentrations were determined from a standard curve constructed using mouse OCN.

Statistical Analysis

Results (mean and standard deviation) were calculated from at least six replicate samples. One-way analysis of variance (ANOVA) was conducted using the computer application InStat (Graphpad Software, San Diego, CA). Post-hoc comparisons were made using the Tukey-Kramer test when the p-value was significant ($p < 0.05$).

RESULTS

Release Profiles

To create intermittent release profiles, drug-loaded microspheres were alternately layered with blank (unloaded) microspheres. Microspheres were 50–150 μm in diameter and had a loading of 68–73 ng simvastatin acid per mg of polymer. By multilayering blank and loaded microspheres, six-, eight-, and ten-layer devices had approximate totals of 3, 4, and 5 μg simvastatin acid, respectively. Figure 1 shows both the designed/predicted and experimentally determined release profiles for 10-layer devices. In general, the shape and duration of the experimental profile were comparable to those of the desired/predicted release profile. Two differences were noted, however. Whereas the designed profile had zero release between the peaks, small amounts of simvastatin acid were measured. In addition, the peak release values were not identical to those predicted in the model. Regardless, the desired intermittent release profiles were obtained.

Figure 2 shows cumulative release of simvastatin acid for the three different blend ratios under dynamic release conditions. All three profiles were linear. The polymer with the highest ratio of CAP to PF-127 (7:3) had a slightly, but significantly ($p < 0.05$), slower release rate. This blend also lasted for a longer time, approximately two days longer. Behavior of the 5:5 and 6:4 blends was statistically similar. And although differences were observed in the rate and duration of release, statistically similar total amounts of simvastatin acid were delivered for all blends.

Figure 3 shows the effect of CAP to PF-127 blend ratio on intermittent release of simvastatin acid under dynamic conditions. All three devices showed exactly five discrete peaks, with each layer degrading over two to three days. As expected from Figure 2, 7:3 blends lasted slightly longer than did 5:5 and 6:4 devices.

The number of layers can be altered to modulate release (Figure 4). Six layer devices had exactly three discrete peaks, eight layer devices had four peaks, and ten layer devices had five peaks under dynamic release conditions. In these experiments, each layer lasted approximately two days, independent of the number of layers.

The release conditions were also found to affect the kinetics of polymer erosion and subsequent release of simvastatin acid (Figure 5). Five discrete peaks were seen when samples were degraded under both static and dynamic conditions. Under static release conditions, however, 10-layer devices lasted for more than 20 days, compared to only 12 days under dynamic conditions.

Cytotoxicity

Degradation products from polymer erosion experiments (*i.e.*, dissolved CAP and PF-127 in the PBS) were diluted in cell culture medium and added to MC3T3-E1 osteoblastic cells (Figure 6). The undiluted release supernatant was considered 100%, and increasing dilutions into the cell culture medium were tested. High concentrations of CAP/PF-127 had a cytotoxic effect, causing cell death. Even 40% degradation products had a time-dependent, adverse effect on the cells ($p < 0.01$). While cell responses were similar within the first two days of exposure, cytotoxic effects became apparent by four days. At 20% and below, however, the cells were unaffected; both the DNA content (reflecting number of cells) and production of alkaline phosphatase (reflecting bioactivity) were statistically similar to levels in control cultures.

Cell Responses to Alternating or Constant Concentration of Simvastatin Acid

Having determined acceptable levels of degradation products, effects of different profiles of exposure to simvastatin acid were investigated. DNA content and alkaline phosphatase (AP) activity in MC3T3-E1 cultures exposed to pulsatile/alternating and sustained/constant delivery of simvastatin acid are shown in Figure 7. In all groups, except 1 μM constant concentration, the amount of DNA increased until the seventh day, and the growth rates were somewhat diminished afterward. Intermittently exposing cells to 1 μM simvastatin acid reduced, but did not eliminate, the adverse effect of this higher concentration. Alternating exposure to lower concentrations of simvastatin acid increased DNA contents in the earlier stage of culture ($p < 0.05$). AP was elevated by intermittent exposure to simvastatin acid. Activity was significantly higher for the 100 pM and 10 nM alternating groups at 10 days compared to the other treatments ($p < 0.05$).

Results for OCN secretion by MC3T3-E1 cells exposed to alternating and constant delivery of simvastatin acid and the controls are shown in Figure 8. In control cultures, OCN levels remained relatively constant at low concentration ($p < 0.05$). At day 1, cells constantly exposed to simvastatin acid showed higher concentrations of OCN compared to controls and close to levels in cells exposed to alternating simvastatin acid. At days 2 and 4, cells constantly exposed to simvastatin acid showed decreased concentrations that then remained fairly constant ($p < 0.05$). For 1 μM constant exposure, levels of secreted OCN were dramatically decreased compared to the 100 pM and 10 nM constant concentrations. All cells intermittently exposed to simvastatin acid showed increased OCN concentrations in the earlier stage of culture ($p < 0.05$), and levels remained high. Concentrations were significantly higher for the 10 nM and 1 μM alternating groups after 2 days in comparison with the other groups ($p < 0.05$).

DISCUSSION

Simvastatin has an HMG-like moiety, which is present as an inactive lactone. *In vivo*, this prodrug is enzymatically hydrolyzed to its active hydroxyacid form (Corsini et al., 1995). Simvastatin also has a rigid, hydrophobic section that is covalently linked to the HMG-like portion. Consequently, the drug has a very low aqueous solubility, approximately 1.4 $\mu\text{g/mL}$ (Serajuddin et al., 1991). More hydrophilic molecules can give a higher local concentration. Therefore, the approach used for enhancing simvastatin solubility was hydrolysis to cleave the lactone ring and convert the molecule to its β -hydroxyacid form (Kaesemeyer et al., 1999).

The hydroxyacid form of simvastatin is approximately three orders of magnitude less lipophilic than the lactone form (Hamelin and Turgeon, 1998).

Cellulose acetate phthalate (CAP) is a cellulose derivative that has been used in the pharmaceutical industry for enteric coating of oral tablets and capsules. While the regulatory status is clear for oral applications (*i.e.*, in FDA Inactive Ingredients Guide), it remains unknown with respect to parenteral devices. However, Heller's group has previously used CAP in formulations for treating heroin addiction (Tefft et al., 1992; Roskos et al., 1993).

Pluronic F-127 (PF-127) is a triblock copolymer of polyethylene oxide and polypropylene oxide. The numerous ether sites within PF-127 allow for hydrogen bonding with carboxylic acid groups in CAP. Blends of CAP and PF-127 form an association polymer that undergoes surface erosion following deprotonation at physiological pH and consequently shows zero-order release.

In contrast to the commonly used biodegradable polyesters, such as poly(lactide-co-glycolide), which undergo bulk hydrolysis, the CAP/PF-127 system degrades by surface erosion, in which the material degrades from the outermost surface toward the inside (Xu and Lee, 1993; Raiche and Puleo 2003). Therefore, the overall shape of the release profiles was linear. When alternating loaded and unloaded layers, flatter regions reflecting erosion of the blank layers were also observed.

As a non-ionic surfactant, PF-127 is readily soluble in water, but CAP is swelled and then relatively slowly dissolved in neutral solutions. Consequently, blends containing higher ratios of PF-127 had shorter duration delivery profiles. But the overall effect was small, in contrast to the work of Lee, in which larger differences in erosion were observed for the different blends (Xu and Lee, 1993; Gates et al., 1994). This discrepancy may be related to structural differences resulting from the fabrication methods. Lee and associates made their devices by simply solvent casting, whereas in the present work, CAP/PF-127 microspheres were made first and then pressure-sintered into multilayered devices. Another reason for the different degradation rates may be the exposed surface area. Lee's samples were exposed on both top and bottom surfaces, so the degradation rate was relatively higher compared to the present devices, which were coated to protect all but one surface from the aqueous environment. Differences in the carboxylate content of CAP obtained from different sources may also have affected material degradation.

Directional control of release was obtained by coating with a more hydrophobic biodegradable polymer. Thus, only one surface was exposed to PBS, and as mentioned previously, the degradation rate was consequently reduced. The total duration of delivery depended directly on the number of layers and the release environment. For each additional set of blank and loaded layers, another release peak was obtained. Even gentle mechanical agitation enhanced erosion of the polymer. These results indicate that, in addition to chemical dissolution effects, physical erosion also plays a role in degradation of CAP/PF-127. Therefore, even though intermittent release will be obtained, the duration of delivery will depend on the site of implantation. Consider, for example, low clearance of erosion byproducts following placement in a relatively confined bony defect compared to an implant more vigorously bathed in body fluids.

Comparison of the present doses to those used clinically is complicated by the significantly different uses of simvastatin: systemic lowering of cholesterol vs. stimulation of local bone formation. The clinically recommended therapeutic dose of simvastatin ranges from 5 to 80 mg/day. With 60 mg dosage forms, serum concentrations of simvastatin and simvastatin acid are 18.7 ± 4.7 ng/ml and 3.5 ± 0.5 ng/ml, respectively. Further, the half-life of simvastatin acid is 5.9 ± 0.3 hr (Ucar et al., 2004). The devices described in the present work released

simvastatin acid at a rate of approximately 36.5 ng/hr into 5 ml of PBS. To stimulate bone formation in animals, Mundy et al. (1999) injected 1–10 mg/kg/day subcutaneously over the calvaria of mice for 5 days. Assuming 30 g animals, up to approximately 300 µg of simvastatin was administered each day. In contrast, the present devices contained up to only 5 µg of simvastatin acid.

As might be expected with many biodegradable/bioerodible materials, high levels of degradation byproducts can be cytotoxic. This has been well-documented for polymers and copolymers of lactic and glycolic acid (Bergsma et al., 1993; Agrawal, et al., 1995; Suganuma and Alexander, 1993; Daniels et al., 1992; Daniels et al., 1994; Edwards et al., 2000; Meir et al., 2000). When biodegradable materials are implanted, however, the concentration of toxic byproducts is reduced via dilution in extracellular fluids and blood and by clearance via the circulatory and lymphatic systems. In the present work, although high concentrations of degradation products were cytotoxic, dilution of CAP/PF-127 byproducts to less than 40% prevented adverse effects. Preliminary results following implantation of CAP/PF-127 release devices in rats show no evidence of adverse effects (data not shown).

The results of DNA content, AP activity, and OCN secretion for preosteoblastic cells exposed to intermittent and sustained concentrations of simvastatin acid at lower concentrations (100pM - 10nM) indicate an anabolic effect. The continued inhibition of HMG-CoA reductase activity and therefore of cholesterol synthesis resulting from high, sustained concentrations of simvastatin acid appear to have adversely affected the cells and prevented growth and activity. After Mundy and colleagues (1999) reported that simvastatin induced expression of BMP-2 in a model reporter system, Maeda et al. (2001) showed that continuous low doses of simvastatin enhanced AP activity and mineralization and increased BMP-2 production in MC3T3E-1 cells.

In the present work comparing alternating exposure with constant treatment, the former stimulated higher osteoblastic activity. This result is similar to that for Mundy's delivery via subcutaneous injection and the resulting local pulsatile simvastatin profile that was found to enhance bone formation. Skoglund and associates delivered simvastatin by daily oral dosing and found that simvastatin-treated mice had a larger transverse area of fracture callus, and the force required to break the bone was greater than controls (Skoglund et al., 2002). Many clinical studies have suggested that statins are related to an increase in bone mineral density and significantly reduced fracture risk (Wang et al., 2000; Chan et al., 2000).

CONCLUSION

Intermittent release of simvastatin acid was achieved using the association polymer system of CAP and PF-127. The release profiles roughly mimic local exposure resulting from repeated oral dosing or subcutaneous injections. Furthermore, the release profiles can be controlled by varying polymer blending ratio, number of layers, and release conditions. Cell number, alkaline phosphatase activity, and osteocalcin secretion were enhanced in preosteoblastic cell cultures treated with alternating concentrations of simvastatin acid. Ongoing studies are directed at evaluating the bioactivity of intermittently released simvastatin acid *in vivo*. Overall, CAP/PF-127 devices can be designed to provide pulsatile release of simvastatin acid, and such alternating concentrations stimulate osteoblastic activity. These devices may be useful for promoting local bone formation.

ACKNOWLEDGMENTS

This work was supported by the Kentucky Science and Education Foundation (KSEF-148-502-03-67) and the National Institutes of Health (AR048700).

REFERENCES

- Agarwal, CM.; Niederauer, GG.; Micallef, DM.; Athanasiou, KA. The use of PLA-PGA polymers in orthopaedics. In: Wise, DD.; Trantolo, DJ.; Altobelli, DE.; Gresser, JD.; Yaszemski, MJ., editors. *Encyclopedic Handbook of Biomaterials and Bioengineering*. Marcel Dekker; New York: 1995. p. 2018-2115.
- Bergsma EJ, Rozema FR, Bos RR, Bruijn WC. Foreign body reactions to resorbable poly (L-lactide) bone plates and screws used for the fixation of unstable zygomatic fractures. *J. Oral Maxillofac. Surg* 1993;51:66–170.
- Boyce T, Edwards J, Scarborough N. Allograft bone: The influence of processing on safety and performance. *Orthop. Clin. North. Am* 1999;30:571–581. [PubMed: 10471762]
- Casey PJ, Seabra MC. Protein prenyltransferases. *J. Biol. Chem* 1996;271:5289–5292. [PubMed: 8621375]
- Chan KA, Andrade SE, Boles M, Buist DMS, Chase GA, Donahue J, Goodman M, Gurwitz J, LaCroix A, Platt R. Inhibitors of hydroxymethylglutaryl-coenzyme A reductase and risk of fracture among older women. *Lancet* 2000;24:2185–2188. [PubMed: 10881890]
- Corsini A, Maggi FA, Catapano AL. Pharmacology of competitive inhibitors of HMG-CoA reductase. *Pharmacol. Res* 1995;31:9–27. [PubMed: 7784310]
- Daniels AU, Taylor MS, Andriano KP, Heller J. Toxicity of absorbable polymers proposed for fracture fixation devices. *Proc. Orthop. Res. Soc* 1992;17:88.
- Daniels AU, Taylor MS, Heller J, Andriano KP. Six bioabsorbable polymers: in vitro acute toxicity of accumulated degradation products. *J. Appl. Biomater* 1994;5:151–157. [PubMed: 10147175]
- Davignon J, Laaksonen R. Low-density lipoprotein-independent effects of statins. *Curr. Opin. Lipidol* 1999;10:543–559. [PubMed: 10680049]
- Edwards CJ, Hart DJ, Spector TD. Oral statins and increased bone mineral density in postmenopausal women. *Lancet* 2000;355:2219–2219. [PubMed: 10881899]
- Gates KA, Grad H, Birek P, Lee PI. A new bioerodible polymer insert for the controlled release of metronidazole. *Pharm. Res* 1994;11:1605–1609. [PubMed: 7870678]
- Hamelin BA, Turgeon J. Hydrophilicity/lipophilicity: relevance for the pharmacology and clinical effects of HMG-CoA reductase inhibitors. *Trends Pharmacol. Sci* 1998;19:26–37. [PubMed: 9509899]
- Istvan E, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science* 2001;292:1160–1164. [PubMed: 11349148]
- Kaesemeyer WH, Caldwell RB, Huang J, Caldwell RW. Pravastatin sodium activates endothelial nitric oxide synthase independent of its cholesterol-lowering actions. *J. Am. Coll. Cardiol* 1999;33:234–241. [PubMed: 9935036]
- Kureishi Y, Luo Z, Shiojima I, Bialik A, Fulton D, Lefer D, Sessa W, Walsh K. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. *Nat. Med* 2000;6:1004–1010. [PubMed: 10973320]
- Labarca C, Paigen A. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem* 1980;102:344–352. [PubMed: 6158890]
- Maeda T, Matsunuma A, Kawane T, Horiuchi N. Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. *Biochem. Biophys. Res. Commun* 2001;280:874–877. [PubMed: 11162604]
- Meir CM, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H. HMG-CoA reductase inhibitors and the risk of fractures. *JAMA* 2000;283:3205–3210. [PubMed: 10866867]
- Mundy G, Garrett R, Harris S, Chan J, Chen D, Rossini G, Boyce B, Zhao M, Gutierrez G. Stimulation of bone formation in vitro and in rodents by statins. *Science* 1999;286:1946–1949. [PubMed: 10583956]
- Popovic JR. 1999 National Hospital Discharge Survey: Annual Summary with Detailed Diagnosis and Procedure Data. *Vital Health Stat* 2001;13:1–206.
- Raiche A, Puleo DA. Association polymers for modulated release of bioactive proteins. *IEEE Eng. Med. Biol. Mag* 2003;22:35–41. [PubMed: 14699934]
- Raiche AT, Puleo DA. In vitro effects of combined and sequential delivery of two bone growth factors. *Biomaterials* 2004;25:677–685. [PubMed: 14607506]

- Roskos KV, Tefft JA, Heller J. A morphine-triggered delivery system useful in the treatment of heroin addiction. *Clin. Mater* 1993;13:109–19. [PubMed: 10146246]
- Serajuddin AT, Ranadive SA, Mahoney EM. Relative lipophilicities, solubilities, and structure-pharmacological considerations of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors pravastatin, lovastatin, mevastatin, and simvastatin. *J. Pharm. Sci* 1991;80:830–834. [PubMed: 1800703]
- Skoglund B, Forslund C, Aspenberg P. Simvastatin improves fracture healing in mice. *J. Bone Miner. Res* 2002;17:2004–2008. [PubMed: 12412808]
- Suganuma J, Alexander H. Biological response of intramedullary bone to poly-L-lactic acid. *J. Appl. Biomater* 1993;15:13–27.
- Sugiyama M, Kodama T, Konishi K, Abe K, Asami S, Oikawa S. Compactin and simvastatin, but not pravastatin, induce bone morphogenetic protein-2 in human osteosarcoma cells. *Biochem. Biophys. Res. Commun* 2000;271:688–692. [PubMed: 10814523]
- Tefft JA, Roskos KV, Heller J. The effect of lipase on the release of naltrexone from triglyceride-coated cellulose acetate phthalate microspheres. *J. Biomed. Mater. Res* 1992;26:713–24. [PubMed: 1527096]
- Thompson GJ, Puleo DA. Effects of sublethal metal ion concentrations on osteogenic cells derived from bone marrow stromal cells. *J. Appl. Biomater* 1995;6:249–258. [PubMed: 8589510]
- Ucar M, Neuvonen M, Luurla H, Dalqvist R, Neuvonen PJ, Mjondal T. Carbamazepine markedly reduces serum concentrations of simvastatin and simvastatin acid. *Eur. J. Clin. Pharmacol* 2004;59:879–882. [PubMed: 14691614]
- Wang PS, Solomon DH, Mogun H, Avorn J. HMG-CoA reductase inhibitors and the risk of hip fractures in elderly patients. *JAMA* 2000;283:3111–3216.
- Xu X, Lee PI. Programmable drug delivery from an erodible association polymer system. *Pharm. Res* 1993;10:1144–1152. [PubMed: 8415399]

Figure 1. Comparison between the desired/predicted and the experimental release profiles for 10-layer devices (7:3 CAP:PF-127).

Figure 2. Effect of blend ratio on cumulative release of simvastatin acid. Data are the mean of at least six replicates. Error bars, which ranged from 3–11% of the mean, are not shown to prevent obscuring the curves.

Figure 3.

Effect of blend ratio on intermittent release of simvastatin acid from 10-layer devices. Data are the mean of at least six replicates. Error bars, which ranged from 5–9% of the mean, are not shown to prevent obscuring the curves.

Figure 4.

Effect of number of layers on intermittent release of simvastatin acid (blend ratio, 7:3). Data are the mean of at least six replicates. Error bars, which ranged from 3–14% of the mean, are not shown to prevent obscuring the curves.

Figure 5. Effect of incubation conditions on intermittent release of simvastatin acid from 10-layer devices (blend ratio, 7:3).

Figure 6.
Effect of CAP/PF-127 (7:3) degradation byproducts on osteoblastic cells.

Figure 7. DNA content and alkaline phosphatase activity for osteoblasts exposed to constant and alternating simvastatin acid.

Figure 8.
Osteocalcin secretion for osteoblasts exposed to constant and alternating simvastatin acid.