A ROBUST METHOD TO COAT ALLOGRAFT BONE WITH A DRUG-RELEASING POLYMER SHELL

Sherry N. Davidoff[1], Brent P. Call,[1] Paul C. Hogrebe,[2] David W. Grainger,[1,2] Amanda E. Brooks[1]
[1]Department of Pharmaceutics and Pharmaceutical Chemistry, Health Sciences Campus, University of Utah, Salt Lake City, Utah 84112
[2]Department of Bioengineering, University of Utah, 72 South Central Campus Drive, Salt Lake City, Utah 84112

ABSTRACT

Bone allograft material used for osseous void filling and structural support in skeletal reconstructive surgeries can also be used in combination as a drug carrier. Previous coating methods to load drugs, such as antibiotics and anti-inflammatories, provided an initial burst release, which may not be optimal for combating persistent local implant-associated bacterial infections. Theoretical drug release kinetics can be optimized not only with a clinically relevant drug-to-polymer ratio but also with a robust, effective rate-limiting release coating method. Three coating methods were evaluated in which degradable polycaprolactone (PCL) polymer retains and controls the release of antibiotic tobramycin from commercial, clinically common allograft bone fragments. Methods are based on a common dip-coating of the allograft fragment, with each coating method distinguished by subsequent drying and processing steps. Using a combination of classic polymer coating techniques, dipping and rapid drying, a method has been developed to apply the drug-releasing polymer coating while concurrently maintaining the high surface area, cancellous pore allograft structure. This provides increased local drug loading and controlled release over the clinically relevant six-to-eight week time period. This method offers potential for industrial scale-up as multiple cancellous allograft fragments can be processed batch-wise. Multiple drugs and combination therapies can also be applied in laminate coating designs.

Keywords: allograft bone, controlled drug delivery system, dip coating, drug loading, methanol flocculation, phase separation, polycaprolactone, orthopedic infection, tobramycin, vacuum drying

INTRODUCTION

More than $1.3 billion dollars of the total orthopedic market is devoted to bone graft-related surgical procedures [1]. Autograft bone (patient harvested bone) is the gold standard for immunologically seamless graft integration; however, patient pain and wound site infection, necessitates another option. FDA-regulated clinically familiar allograft fragments (cadaveric sourced bone) offer an acceptable alternative in the context of integration and structural support. Unfortunately, regardless of the source, pathological infectious events remain common to bone grafting of all kinds, occurring in 1-3% of the more than 500,000 surgeries performed in the U.S [2]. Although local, controlled drug release is not a novel concept, direct delivery to the bone presents an additional pharmaceutical and kinetic challenge due to the limited pharmaceutical penetration into bone that constrains systemic administration efficacy [3]. An understanding of biological systems, incorporation and controlled release of bioactive agents to maximize the temporal local therapeutic effects [4] is gaining popularity. This next generation in biomaterials initiates and directs

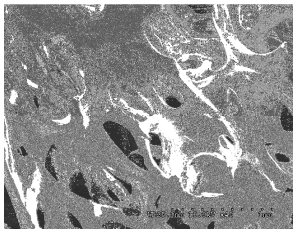

Figure 1. SEM image of cancellous allograft fragment.

Presented at Rocky Mountain Bioengineering Symposium &
International ISA Biomedical Sciences Instrumentation Symposium
9-11 April 2010, Laramie, Wyoming, www.isa.org

specific cellular responses to accommodate the implant and accelerate integration and healing with engineering precision and drug-mediated bioactivity. The high porosity of cancellous allograft fragments (Figure 1) imparts a large surface area and an appropriate three-dimensional architecture to be developed into a drug delivery device preventing complex orthopedic infections.

Device surface properties, long recognized as an important component in improving the performance of materials interfaced with tissue or biological milieu, can be tailored to contain therapeutically relevant biological compounds. However, technical application of a coating often complicates and diminishes the efficacy of local drug delivery. Dip coating allograft bone presents a dichotomy that must be balanced. Although dip coating with a polymer overcoat is a scalable, economic, and routine coating technique, it potentially suffers from delamination, coating fractures during drying, and occlusion of the allograft porous structure. A multi-step dip-coating procedure in which the material is submerged into the carrier/antibiotic solution multiple times, and allowed to dry between subsequent "dips", is a common approach. Layer-by-layer assembly of surface coatings and other encapsulation techniques offer a promising strategy for time-dependent controlled drug release and burst release control [5]. Dip coating, in conjunction with vacuum quick drying potentially maintains the pore structure of the material but can also trap air and solvent bubbles within the coating structure leading to inconsistencies in the coating and a "percolation-type" release effect. Alternatively, the pore structure may be maintained to increase drug loading via methanol flocculation. This method forces loose polymer to be excluded from the cancellous structure; though this too results in an inconsistent and unknown drug loading. Ultimately, despite its promise, the procedure to "load" the drug on the allograft bone and release it over a clinically relevant time period positions this technology just beyond the grasp of the commercial market.

Antibiotic-coated surfaces have been employed for the past 2 decades with a great deal of success; however, the release of the antibiotic at low levels over time limits efficiacy and potentially can lead to the development of antibiotic-resistant bacterial strains. Thus, although they have the benefit of targeted drug delivery, their use does not effectively address the problem of hospital-acquired infections. Delivery must be controlled to maintain the drug in therapeutic concentration ranges (i.e. above the minimal inhibitory concentration and below the cytotoxic concentration) over a target time period. The rate of drug release can be altered using a common biodegradable polymer as a releasing agent. We are currently developing an allograft-based, drug-releasing technology for local antibiotic delivery to orthopedic sites in the operating theater. This new drug-releasing technology employs clinically familiar polycaprolactone (PCL) as the polymer carrier because it possesses molecular weight dependent biodegradable properties to control the release of tobramycin from allograft bone fragments. Importantly, tobramycin exhibits temperature stability and broad spectrum activity [6] appropriate for orthopedic infections. The system initially appears simplistic, but in practice, developing an understanding of the physicochemical interactions between the polymer and the antibiotic and the impact of coating technique on the release kinetics is critical to the design of such a system. Assessment of coating integrity and release kinetics based on multiple coating techniques provides objective criteria to evaluate and select the most effective technique for future development.

METHODS

Sample Fabrication: Cancellous allograft bone croutons (Miami Tissue Bank) were massed and like size fragments were selected for each batch. Polycaprolactone (PCL, 10 kD) was dissolved at 100 mg/mL in acetone at 45˚C. Tobramycin was added to the PCL solution as a 10% mass/volume ethanol solution. Each bone crouton was dip coated in the tobramycin/PCL solution and dried via methanol flocculation, vacuum drying, or air/heat drying. Each crouton was massed and the procedure was

repeated to obtain a coating of approximately 20mg of tobramycin containing PCL. Subsequently, a 10kD PCL unloaded (no drug PCL solution) overcoat was applied in part of the cohorts (half of the croutons from each drying technique) and dried according to the same method as it was originally processed. Every crouton was massed again after application of the unloaded overcoat.

Methanol Flocculation: Methanol was filtered through the porous structure of some allograft cohorts (n=10) immediately following dip coating, precipitating the PCL from solution and removing excess, unbound PCL from the crouton..

Vacuum Drying: After dip coating, certain cohorts of croutons were placed in a vacuum flask and placed under vacuum pressure for approximately 3-5 minutes to quick dry the PCL acetone solution to the porous structure of the cancellous allograft crouton (n=12).

Air/heat drying: After dip coating, certain cohorts of croutons were allowed to dry on a sandbath for 15 minutes at 48°C, (n=10).

Scanning Electron Microscope (SEM) Imaging: Two croutons from each cohort (vacuum dried with or without an unloaded overcoat, methanol treated with or without an unloaded overcoat, air dried with or without an unloaded overcoat) were used for SEM (Hitachi S-3000N, Pleasanton, CA) imaging. Each sample was spattered coated with gold particles for approximately 4 to 7 minutes. Link Isis series 300 microanalysis system software displayed the real-time images captured by the microscope and allowed the capture of images varying between 1mm to 500 micron magnification. Pore and fracture size was measured with PCI.

Tobramycin Release Kinetics: Three croutons from each cohort were individually submerged in 5mL of phosphate buffered saline (PBS) and incubated at 37˚C. PBS was collected at 6 time intervals: 30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, and 24 hours. Tobramycin concentration was determined using a modified o-phthaldialdehyde (OPA)-based fluorescence assay (100 µl of sample, 100 µl of isopropanol, and 200 µl of OPA reagent (Sigma P-0532) incubated for 30 minutes at room temperature and read at excitation 360nm and emission at 460nm [7] using a microplate reader (Biotek spectrophotometer and GenePix5 software). A fresh sample of PBS was added at each time point.

Data analysis: Concentrations and percent released were calculated based on a linear regression of tobramycin standards and the amount of tobramycin applied to each crouton system determined by the mass of the drug-PCL coating. Concentrations were plotted and one-way ANOVAs were used to determine statistical differences. Limits of detection for the assay were determined using based on an extensive set of tobramycin standards and an optimized linear regression.

RESULTS

Macroscopically, all techniques seemed to provide approximately the same level of coating porosity; however, to determine coating integrity, scanning electron microscopy (SEM) was used. SEM imaging also showed no remarkable difference in observed pore sizes based on drying methods but revealed fractures in the coating emanating from the pores (Figure 2). Compared to the other methods, air dried croutons displayed 1) a pore structure occluded with drug and polymer, 2) qualitatively less drug and polymer aggregated on the surface, and 3) limited surface fractures. Additionally, all images show what appears to be drug and polymer concentrated at the surface, with a surprisingly greater amount shown on samples with an additional unloaded 10kD PCL overcoat (Figure 2A and 2C).

It was suspected that microscopic inconsistencies may impact the drug's burst release; therefore, short-term burst release kinetics were determined to assess the impact of drying and processing conditions (Figure 3). Burst release, although often viewed as a hindrance for long-term controlled release systems, is necessary to combat wound-site pathogenic bacteria by rapidly achieving a local drug concentration above the minimal inhibitory concentration; however, burst release kinetics must also be controlled. In an attempt to modulate drug burst release, an additional 10kD PCL unloaded overcoat was applied. This coating was hypothesized to control surface-blooming of the drug. OPA was reacted with tobramycin released from the croutons into PBS, and average fluorescence intensity was compared. For the cohorts processed by air-drying and methanol flocculation, no significant difference between having or lacking the additional overcoat was observed (Figure 3A, 3B) with the exception of methanol processed croutons at 8 hours (α=0.05, $p<0.01$) and air-dried at 8 hours (α=0.05, $p<0.03$) and 24 hours (α=0.1, $p<0.07$). Conversely, when croutons were vacuum-dried, addition of an unloaded overcoat greatly slowed the tobramycin release out to 24 hours (Figure 3C). At 30 minutes there was a significant difference in a coated versus uncoated crouton (α=0.05, $p<0.0007$). Significant differences were also seen in the remaining time points, (α=0.1, 1hr: $p<0.002$, 2hr: $p<0.02$, 4hr: $p<0.006$, 24hr: $p<0.02$) with the exception of 8-hours. Alternately, drug burst release was modulated by the fragment processing conditions (i.e., air drying, vacuum drying or methanol flocculation). At 1 hour, 2 hours and 24 hours there was no significant difference between processing methods of croutons *with* an additional unloaded overcoat; however, there was a significant difference (α=0.1) between vacuum drying and air drying at 30 minutes ($p<0.06$), 4 hours ($p<0.1$) and 24 hours ($p<0.06$). At 8 hours there was an observed significant difference between methanol flocculation and air drying (α=0.1, $p<0.06$) as well as vacuum drying (α=0.1, $p<0.003$). For croutons *without* an overcoat there was no

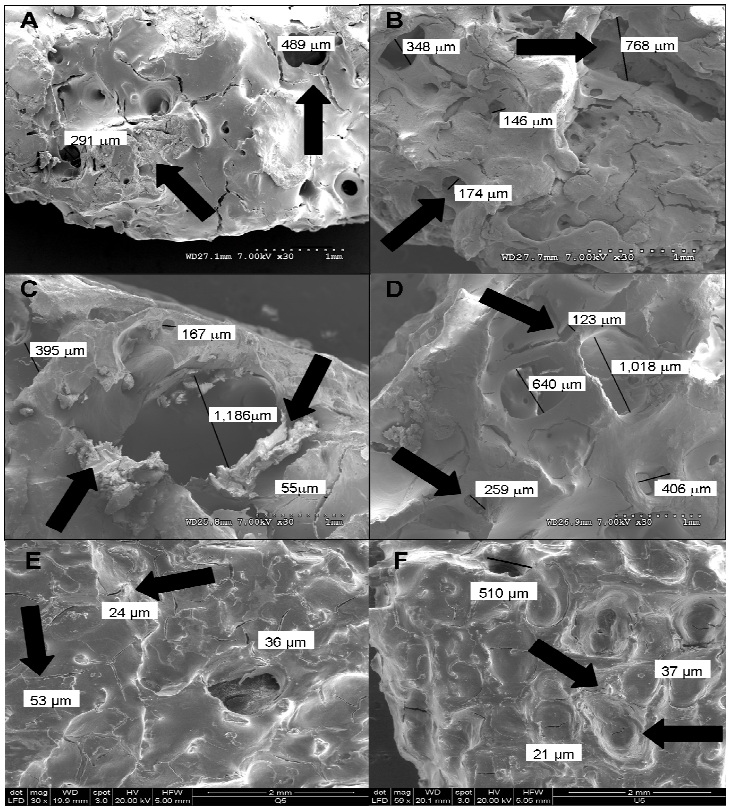

Figure 2. Vacuum dried allograft bone croutons coated with tobramycin/PCL and A) an unloaded 10kD PCL overcoat or B) without an unloaded overcoat. Arrows indicate i) the collection of polymer/drug on surface and ii) cracks around the allograft pore. Notice the wide variation in pore sizes. Methanol treated allograft croutons coated with tobramycin/PCL and C) an unloaded 10kD PCL overcoat or D) without an unloaded overcoat. Arrows indicate i) the collection of polymer/drug along the outside edge of the pores and ii) collection of cracks and material around the pore. Notice the size of fractures relative to the pores. Air dried allograft bone crouton coated with tobramycin/PCL and with E) an unloaded 10kD PCL overcoat or F) without an unloaded overcoat. Arrows indicate the varying crack sizes on the coat surface. Notice the compromised pore structure.

significant difference between any of the methods at 2 and 24 hours. There was a significant difference (α=0.05) between methanol flocculation and vacuum drying at 30 minutes ($p<0.03$) and 1 hour ($p<0.04$). At 4 ($p<0.02$) and 8 ($p<0.01$) hours, differences (α=0.05) were observed between methanol flocculation and air drying. Interestingly, vacuum and air drying only showed a significant difference at 8 hours (α=0.05, $p<0.03$).

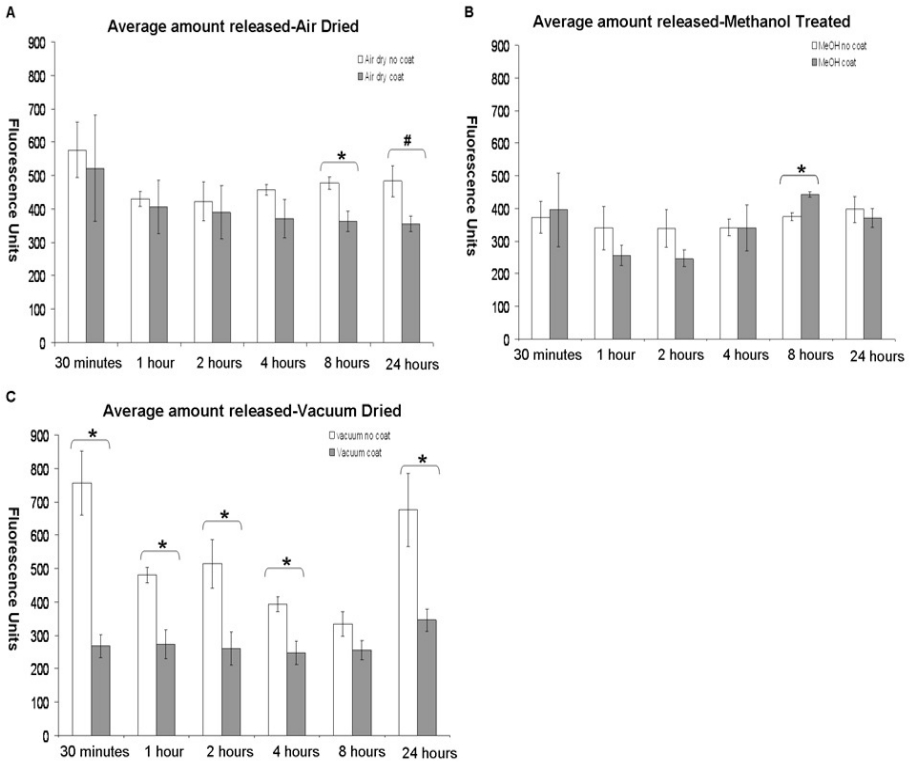

Figure 3. Comparison of the average amount of tobramycin released per processing and coating conditions. Each graph depicts allograft fragments that have an unloaded overcoat (shaded coloring) with those lacking the overcoat (black). Different treatment conditions are shown in A (air dried), B (methanol treated), and C (vacuum dried). * indicates a significant difference at α=0.05 while # indicates significance at α = 0.1.

DISCUSSION

SEM imaging of polymer-coated croutons treated with different drying conditions does not distinguish each method. Each method produced fragments with fractured surfaces (Figure 2A, 2C) indicating that both methanol flocculation and vacuum drying rapidly and forcibly remove the solvent, disrupting the polymer matrix coat. During air drying the acetone solvent slowly evaporated from the coating, limiting surface fractures while potentially allowing more extensive phase separation (Fig. 2E and 2F) as evidenced by SEM images. (Fig. 3A). In addition to the allograft pore structure that improves drug loading capacity, small pores become evident in the surface coating as the acetone solvent is rapidly excluded from the polymer network. Each of these "coating pores" compromises the integrity of the coating, creating a concentration of stress evidenced by clusters of imperfections surrounding the allograft pore (Fig. 2) as well as a pathway that allows biological milieu unencumbered access to a potentially interconnected network of tobramycin molecules. The antibiotic exhibits incompatible miscibility with the polymer, and is affected by the concentration ratio of polymer to drug. Phase separation of the tobramycin resulting from variation in the miscibility of the system is reflected in the accumulation of the drug or polymer (Figure 2A, 2C) on the surface of the allograft fragment despite the processing technique. A comparison of the measured pore sizes initially suggests a difference in the coating techniques; however, pore size is a fallible basis for comparison due to inconsistencies in the initial pore size and structure of each cancellous allograft crouton not reflected in the measured mass of the fragment. SEM imaging reveals coating inconsistencies and/or phase separation as a logical explanation for tobramycin burst release.

Previous coating methods that varied the molecular weight, and consequently the degradation of the PCL showed only an initial burst release of antibiotic, independent of the polymer shell; this is not optimal for formulating the drug as it appears to be a strong indicator of phase separation. Alternatively, it is more desired and clinically more relevant to engineer a polymer-controlled delivery system that provides an initial burst release below the antibiotic's toxic concentration followed by sustained antibiotic levels that remain above the minimal inhibitory concentration (MIC). Burst release often occurs within the first 24 hours in our antibiotic-loaded allograft system and is thought to either stem from coating imperfections or phase separation; thus, the kinetics can potentially be altered using an unloaded polymer overcoat or altering post-coating processing, or changing polymer-drug miscibility. Assessment of release kinetics based on either overcoat application or drying technique reveals that burst rate can be altered using a combination of unloaded polymer overcoat and a vacuum drying technique (Figure 3). Surprisingly, there was no significant effect of an unloaded overcoat with either the air drying or methanol flocculation methods. Processing methods did not impact the kinetics as initially anticipated; however, in light of coating imperfections and phase separation observed in the SEM images (Figure 2), this is not surprising. Importantly, all processing methods maintained tobramycin levels above the minimal inhibitory concentration while staying below the toxic concentration out to 4 weeks (data not shown) indicating the efficacy of our polymer-controlled antibiotic delivery system.

CONCLUSIONS

Drug-polymer interactions within a coating process (i.e., concentration, solvent conditions, non-drug containing overcoat, etc.), drug (concentration, solvent, etc.) and structural support materials (cancellous fragments, micron-sized allograft particulate, etc.) change the coating morphology and resulting antibiotic delivery rate. Alternative methods that exclude all solvent from the system (i.e. neat) will be considered to control phase separation and the release percolation threshold. Overall, allograft bone has been shown to be an effective tool for the local delivery of tobramycin with the release kinetics controlled not only by the polymer matrix but also by the application and processing techniques; however, more work remains to be done to optimize the release and maintain the bulk porosity.

ACKNOWLEDGMENTS

Justin Sevy is thanked for running the OPA fluorescence assay. This work was funded by the University of Utah Technology Commercialization Office.

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