International Journal of Biomedical Materials Research
2013; 1(2): 21-25
Published online January 10, 2014 (http://www.sciencepublishinggroup.com/j/ijbmr)
doi: 10.11648/j.ijbmr.20130102.11

Comparisons of release of several antibiotics from antimicrobial polymer-coated allograft bone void filler

Benjamin D. Brooks[1], Sherry N. Davidoff[2], David W. Grainger[1, 2], Amanda E. Brooks[1, *]

[1]Department of Pharmaceutics and Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT, USA
[2]Department of Bioengineering, University of Utah, Salt Lake City, UT, USA

Email address:
amanda.brooks@utah.edu(A. E. Brooks)

To cite this article:
Benjamin D. Brooks, Sherry N. Davidoff, David W. Grainger, Amanda E. Brooks. Comparisons of Release of Several Antibiotics from Antimicrobial Polymer-Coated Allograft Bone Void Filler. *International Journal of Biomedical Materials Research.* Vol. 1, No. 2, 2013, pp. 21-25. doi: 10.11648/j.ijbmr.20130102.11

Abstract: Osteomyelitis remains a significant complication in orthopedic surgeries. Although infection rates remain steady at 1-3% for primary orthopedic surgeries, overall numbers of orthopedic procedures are increasing, corresponding to earlier and more frequent surgical intervention for an active, aging population. To address this dangerous surgical complication, degradable polycaprolactone (PCL) polymer/antibiotic solutions were coated over allograft bone void filler. Local in vitro release of ciprofloxacin, vancomycin, oxacillin, tobramycin, or rifampicin from a polymer-controlled, antibiotic-releasing bone graft void filler and monitored in vitro allowed the criterion for successful local antibiotic-releasing devices to be expanded. Although each antibiotic exhibited a different release profile based on their formulation and chemical structure, allowing the potential for engineering combinatorial therapy with microbicidal activity, bacterial killing activity in vitro was demonstrated efficacious out to a clinically relevant 8-week time point. In addition to proposing an expanded criterion for successful local antibiotic-releasing devices, this study demonstrates that allograft bone can act as a local, controlled drug release matrix in bone sites. This combination device provides osteoconductive potential in bone voids while mitigating the potential for operatively sourced opportunistic infectious complications during orthopedic repairs as well as primary and revision arthroplasties

Keywords: Controlled Release, Antibiotic Delivery, Orthopedic Infection, Antibiotic Combination Therapy, Osteomyelitis, Bone Void Fillers, Antimicrobial Allograft, Polycaprolactone

1. Introduction

Over 3 million orthopedic surgeries are performed annually in the United States requiring use of bone graft, including cadaveric-sourced allograft, autografts, and synthetic biomaterials as bone void fillers (1). Despite recent materials and surgical advances, infection rates in total joint replacement surgery remain steady, ranging from 1-3%, with recurrence rates in revisions from 8-15% (2); nevertheless, absolute numbers of patients suffering with acute or chronic osteomyelitis are rising from increasing numbers of implant recipients, lifetime risk of implant bacterial seeding, finite infection rates, and improved microbial detection methods (3).

Osteomyelitis is primarily attributed to *Staphylococcus aureus* (4) colonization of a traumatic or surgically induced wounds, bone defects or implant sites. Standard of care treatment includes traditional systemic antibiotic regimes associated with implant removal and/or tissue debridement, bone void fillers adsorbed with antibiotics (i.e., as off-label applications), antibiotic-infused collagen sponges, or antibiotic-impregnated bone cement (5). Unfortunately, while these approaches exploit certain advantages of local antibiotic delivery, they lack controlled pharmaceutical release strategies necessary for effective local dosing and duration. Poor control can promote conditions that facilitate development of antibiotic resistance and continuing infection. According to the U.S. FDA, the potential that a drug-releasing medical device may result in antimicrobial resistance is a “serious clinical problem” (6). Kelm et al. outlined a set of criteria for selecting antibiotics for localized polymer-mediated orthopedic delivery include: (1) available solid dosage form, (2) “wide antibacterial spectrum”, (3) bactericidal potency low concentrations, (4) “elution from [polymer] in high concentrations for prolonged periods”, (5) “low or no risk of allergy or delayed hypersensitivity”, (6)

"low influence on the mechanical properties of the cement", and (7) "low drug serum protein binding" (7). Some of these criteria are fulfilled by the inherent nature of the incorporated antibiotic.

Ideal local antibiotic delivery systems should provide reliable pathogen killing via an immediate burst release within the first 24-hour period after administration followed by sustained drug release above the tissue bed's minimal inhibitory concentration (MIC) to address the remaining microbial threat out to a 6-to-8-week time point (Figure 1A) asserted by the orthopedic community as an important point for infection prevention (8). Several antibiotic-releasing systems are currently marketed as bone void fillers including calcium sulfate based products (Osteoset T® and Herafill G®), PMMA carriers (Septopal, Spacer G, Vancogenx®), and collagen fleece carriers (Collatramp G® and Septocoll®) (9). These products release antibiotic over short periods (usually one to two weeks) and may degrade quickly (9). Bone grafts with extended drug release in the clinically relevant 8-12 week post-operative timeframe that are also osteoconductive are not reported (4). A longer drug release profile with similar capability for bone integration could potentially reduce the incidence of osteomyelitis.

Specifically, use of allograft bone as a drug delivery vehicle with extended rate-controlling capabilities, along with defined degradation and bone remodeling has not been reported, especially for drug release extending beyond a few weeks. This design provides antimicrobial dosing, bioactivity and a level of delivery control unattainable with drug physisorption or soaking reported previously (10,11). Although polymer-controlled local drug delivery to bone is not a new idea (12) successful tailoring of the polymer rate-controlling barrier to provide predictable extended drug release bioactivity coordinated with osteoconduction improves this classic concept (10,11). The impact of drug solubility and drug-polymer miscibility on bioactivity release profiles from this construct have been studied: water-soluble antibiotics (vancomycin, oxacillin, tobramycin) and water-insoluble antibiotics (ciprofloxacin, rifampicin) were formulated into degradable PCL coatings applied to bone allografts, using either organic solvent/water non-solvent binary solutions or pure organic solutions, and released in vitro. Tailoring antibiotic release bioactivity using antibiotic-polymer miscibility provides extended, antibiotic delivery that can be customized and combined to meet established criteria and accommodate diverse clinical needs while better addressing antibiotic-resistance

2. Materials and Methods

2.1. Fabrication of Antimicrobial Allograft Bone Fragments. Antimicrobial Allograft Bone Fragments

Antimicrobial Allograft Bone Fragments (AABF) were fabricated as previously described [8] with the exception that a variety of different antibiotics (ciprofloxacin, rifampicin, oxacillin, vancomycin, and tobramycin) were used. Briefly, PCL (MW = 10kD) was dissolved in 45°C acetone. Individual drugs were added as a bulk powder at 10% w/w. In certain formulations, a 4% water non-solvent was added to the PCL solution prior to the addition of drug for solubilization. Cancellous allograft bone fragments were weighed, dip-coated into PCL drug solutions, dried and subsequently weighed again to determine drug/polymer loading for each crouton. Scanning electron micrograph (SEM) images of coated samples were taken as described in supplementary methods.

2.2. Drug Release Studies

Release studies were conducted as previously described (10,11) with the exception that AABF samples were released into 5mL of 10 mM Phosphate Buffered Saline (PBS) at 37˚C.

2.3. Bacterial Killing

Zones of inhibition (ZOI) were determined on BHI (Brain Heart Infusion) agar plates using *S. aureus* (strain ATCC 25923) according to standardized CLSI methods (13).

3. Results

3.1. AABF Fabrication and Analysis

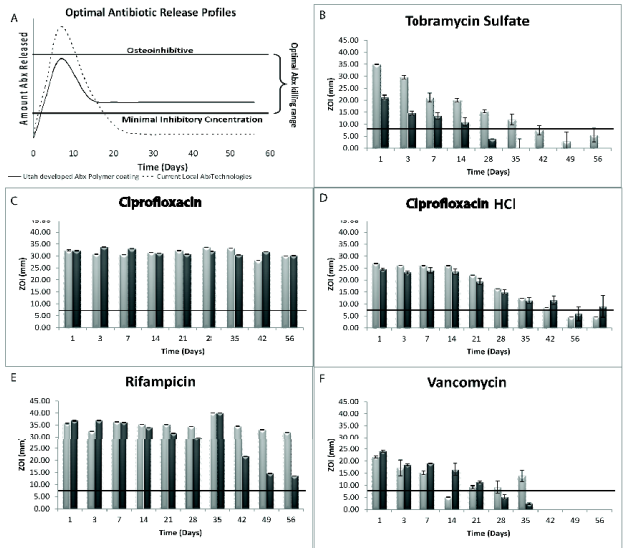

Figure 1: *Zone of inhibition (ZOI) studies using S. aureus agar cultures for different antibiotic drugs released from antimicrobial polymer-coated bone allograft. (A) Conceptual representation of an optimal local release profile for antibiotics where the released drug concentration is sustained above the drug's MIC for the pathogen and below the local tissue toxic concentration that inhibits osseo-integration over extended time in vivo; (B-F): Gray bars represent organic solvent drug loading in the PCL polymer coating on allograft; black bars represent binary solvent/water non-solvent drug loading preparation of polymer-drug coating on allograft. ZOI for: (B) tobramycin (C) ciprofloxacin free-base, (D) ciprofloxacin salt form, (E) rifampicin, and (F) vancomycin. Horizontal line on graph at 8 mm indicates disk size.*

Drug solubilized within the PCL solution was coated over cancellous bone croutons. SEM images of polymer-coated AABF croutons exhibited coating continuity, uniformity and consistency (Suppl. Fig. 1). Addition of a water non-solvent produced coatings that appeared to exhibit microporous character; whereas, the organic solvent coating maintained a minimal open porous structure of the underlying cancellous allograft bone substrate structure and contour. Despite these negligible differences, drug bioactivity remained statistically consistent between the organic and the water non-solvent coating formulations.

3.2. Antibiotic Release Bioactivity

Bioactive antibiotic release was determined based on ZOI assays utilizing the in vitro release media (PBS) (Fig. 1), which decreased over time as expected. In order to examine differences in released drug bioactivity profiles over time due to the solubility of the drug, the raw form of the antibiotic ciprofloxacin (free-base) was incorporated into the polymer coating formulation and applied to cancellous allograft substrates. Both forms of the drug show strong killing although that for the free-base form extends to longer periods of time. Interestingly, the non-salt antibiotic formulations (ciprofloxacin and rifampicin) showed enhanced ZOI compared to antibiotic salts (vancomycin, ciprofloxacin HCl, and oxacillin). Although oxacillin was incorporated into certain formulations, data are not shown (due to bacterial killing efficiency and inconsistent elution kinetic and ZOI data correlations). These problems were unique to oxacillin and may be an artifact of the ZOI assay itself. Inhibition of microbial growth for formulations that incorporated a non-salt antibiotic into a purely organic solvent system correlated with apparent zero-order release kinetics as indicated by no significant loss of bioactivity through the end of the study (8 weeks) (Figure 1C and 1E).

In addition to considering the impact of the raw drug form, supplementing the polymer antibiotic formulation with a water non-solvent component may enhance antibiotic solubility, providing improved release control. As previous publications indicated, water as a non-solvent system affected drug release kinetics (11); however, the addition of water as a non-solvent to the polymer-drug formulation impacted drug release and ZOI very little in this study with the noted exception of reduced killing efficiency at later time points (Figure 1).

4. Discussion

Infection rates in orthopedic surgeries might be reduced through improved local delivery of antibiotics at surgical sites (5). Localized antibiotic delivery typically provides substantial antibiotic release directly to tissue sites within several days to kill resident pathogens, minimizes systemic toxicity, and ensures initial drug concentrations above the MIC at the implant site.[4,7] Most common vehicles for localized drug delivery to bone include non-degrading antibiotic-releasing bone cements, which utilize gentamycin, tobramycin, or vancomycin bulk-loaded into a non-porous poly(methylmethacrylate) (PMMA) glassy matrix.[13-15] These biomaterials are commonly used in revision surgeries to secure implants into bone, but are not approved as bone defect fillers. Unfortunately, these bone cements do not meet some of the relevant regulatory and clinical criteria for use as a local antibiotic-eluting device and permanent foreign body implants, specifically, they do not display bactericidal potency at low concentrations, “elution from [polymer] in high concentrations for prolonged periods”, and have a “low influence on the mechanical properties of the cement”; while other criteria are met by the nature of the incorporated antibiotic (Table 1). Aminoglycosides (e.g., tobramycin and gentamicin) and glycopeptides (e.g., vancomycin) meet most of these criteria (7). Most antibiotic-loaded bone cements incorporate a substantial antibiotic load; however, loaded drug dose release is far from complete (estimated only 10-40% availability with the remainder trapped within the PMMA matrix (14)), dropping tissue concentrations below the MIC after 1-2 weeks (14). This release profile allows infection to occur and also promotes antibiotic resistance and biofilm formation (14). While infection rates are reduced with antibiotic-releasing bone cement, concerns regarding induction of antibiotic-resistant pathogen strains are increasing (5) in particular with gentamicin in PMMA cement (15). Thus, development of degradable polymer-controlled antibiotic-releasing bone void fillers, especially bone allograft that remodels under osteoconductive influences, should prompt some re-evaluation of these criteria and antibiotic requirements for clinical efficacy.

Two new additional criteria, namely (8) minimal inhibition of host osseointegration and (9) reduced induction of microbial antibiotic resistance, better complete specific requisite properties for polymer antibiotic-release bone void fillers. While the previous criteria are related to either the polymer matrix or the incorporated antibiotic, the efficacy of any drug release system must also consider the interaction of the system components as well as the foreign body response [8]. This is embodied by the two additional criteria proposed. Table 1 provides known physical properties for antibiotics considered here related to these criteria. While tobramycin, gentamicin, and vancomycin remain central clinical candidates, other antibiotics warrant consideration based on their release profiles and “bactericidity”, especially ciprofloxacin and rifampicin. Thus, combination devices created by incorporating antibiotics into biomedical degradable polycaprolactone polymer coatings on clinically familiar bone void fillers (10,11) seek to 1) fill dead spaces and facilitate osteoconduction in orthopedic surgical repairs, 2) prevent surgical osteomyelitis, and 3) mitigate possible microbial antibiotic resistance -- all necessary for effective treatment and healing.

Past studies releasing tobramycin from the described polymer-loaded drug delivery system suggest that

modulation of polymer and graft-coating techniques provides versatility to fabricate combination approaches to minimize bone infections (10,11). The current study demonstrates that the solid formulation of the antibiotic, either as an antibiotic salt or its free-base form, alters drug loading and release profiles based both on polymer and drug solubility, and solvent/solute miscibility parameters. Localized, sustained drug release and pathogen killing observed throughout the 8-week study end point significantly advances orthopedic infection control possibilities. Based on these antibiotic-specific release profiles, future development of this approach may consider combination in vivo therapies that reduce orthopedic infection risks in tandem with both passive and active enhanced osteo-conduction and induction strategies, and also that minimize antibiotic resistant selection pressures by using two or more antibiotics. Furthermore, all future localized polymer-controlled drug-releasing implants should abide by the current criteria (vida supra) as well as the two additional proposed criteria to provide optimal infection prevention and osseo-integration.

Table 1. *Antibiotic properties considered to be important for local delivery for orthopedic applications based on currently accepted criteria for local antibiotic-releasing devices (7).*

Drug/ Drug Class	Molecular Weight	Melting Temp (°C)	Water solubility (mg/ml)	Antibacterial spectrum	Allergy risk	Serum protein binding	Bactericidity MIC of *S. aureus* (mcg/ml)	Susceptibility to development of Abx resistance	Available forms
Ciprofloxacin (Quinolone)	331.4	255	Insoluble (0.001)	Broad Spectrum	+	16-43%	0.12 - 2	+	Salt, Free-base
Gentamycin (Aminoglycoside)	477.6	102-108	Soluble (100)	Gram-positive and Gram-negative	+	<15%	0.5 - 2	+	Salt
Oxacillin (Beta-Lactam)	401.4	180	Slightly soluble (13.9)	Enteric bacteria and other eubacteria	+++	89-94%	2	+++	Salt
Rifampin (Rifamycin)	823	183-188	Slightly soluble (1.4)	Gram-positive and fastidious Gram-negative bacteria, Mycobacteria	+	87-91%	0.01-0.1	+	Free-base
Tobramycin (Aminoglycoside)	467.5	168	Highly soluble (538)	Gram-negative	+	<30%	0.24 – 1	+	Salt
Vancomycin (Glycopeptide)	1449.3	185-188	Soluble (100)	Mainly Gram-positive bacteria, Mycobacteria	+	30-60%	0.5 – 2	++	Salt

Acknowledgements

J. Sevy and J. DeGooyer are recognized for technical support and Dr. B. Evans is thanked for providing allograft bone and consultation.

Funding

This work was supported by the University of Utah Technology Commercialization Office, the University of Utah's office of Undergraduate Educations and Elute, Inc. (Salt Lake City, UT, USA).

Transparency Statement

Authors AEB and DWG also have a financial interest in Elute, Inc., Salt Lake City, UT, USA. Authors BDB, DWG, and AEB hold the patent on the drug release technology described herein and licensed from the University of Utah

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