POLYMER-CONTROLLED EXTENDED COMBINATION RELEASE OF SILVER AND CHLORHEXIDINE FROM A BONE VOID FILLER

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ABSTRACT

Although rates of total joint prosthetic infections remain relatively constant at 1-3%, an increasing number of orthopedic procedures and a corresponding rise in the absolute number of infectious complications mandate distinctly new solutions. In order to combat the implant infection threat, an antibiotic-releasing bone void filler (BVF), commercial tradename, ElutiBone™, has been developed using a combination of commercially available ceramic-based BVF plus clinically familiar biocompatible polymers, and a variety of select, dispersed antibiotics. While several traditional antibiotics have been successfully released for an extended duration, a more versatile strategy, releasing multiple antibiotics simultaneously, may be possible. In this study, the antiseptic chlorhexidine and a variety of bacteriostatic silver compounds were incorporated to provide synergistic antimicrobial activity upon release in combination formulations from ElutiBone matrices. Silver chloride was the most effective bacteriostatic tested ($p \leq 0.05$), showing a measurable zone of inhibition at spiked concentrations as low as 31µg/ml. Subsequently, silver chloride was used in combination with the antiseptic chlorhexidine to test for enhanced antimicrobial bioactivity against *S. aureus*. Measurable synergy between the two compounds confirmed the suitability of ElutiBone™ to locally deliver this multidrug antimicrobial cocktail. A myriad of other drug interactions could and should be tested in this novel system in order to expand the utility and combat the increasing prevalence of polymicrobial infections.

Keywords: controlled release, antibiotic delivery, orthopedic infection, antibiotic combination therapy, antimicrobial allograft, polycaprolactone, osteomyelitis, combination device

INTRODUCTION

Nearly 1.3 million people in the United States received a joint prosthesis in 2010, representing a steady increase in total joint replacement (TJR) procedures over the past decade. This increased demand in conjunction with finite infection rates (1-3%) [1], and better microbial detection methods have culminated in higher absolute numbers of patients suffering with acute or chronic implant infections [2]–[5]. Furthermore, approximately 10% of primary TJRs require a revision surgery wherein recurrent infections are reported at rates 20-30%, with secondary infections running nearly 18% [6]–[8]. Three recognized primary causes for these recurrent infections are: 1) lack of device stability [9]; 2) bone's limited vascular supply, and 3) sequestra — localized areas of ischemia and necrosis that present a favorable, inert environment for harboring bacteria and allowing their unmitigated persistence in the dysvascular wound space [10], [11]. Moreover, the economic burden for surgically addressing periprosthetic infections (PIs) with revision TJRs is calculated to be 5.3-7.2 times higher than that of primary TJR operations [12]. Current clinical tools to address acute and chronic bone infection are invasive, costly, and frequently ineffective, further compromising the patient's overall health and recovery [12], [13].

Clinical approaches for treatment or prevention of PI fall into two groups, often administered simultaneously. Systemic antibiotic prophylaxis is considered the standard of care; however, studies are lacking to support this approach to PI treatment [12], [14]. While considered generally effective,

problems with systemic antibiotic delivery include systemic side effects and low antibiotic concentration at bone infection sites — potentially promoting antibiotic resistance [12], [15]. The second treatment option involves localized delivery of antibiotics directly to the site of infection. This strategy is commonly embodied by 1) surgical debridement with an antibiotic solution [16], 2) application of antibiotic solutions to bone grafts by soaking in high concentration antibiotic solutions, and 3) implantation of antibiotic-loaded bone cements. Even though surgical debridement with antibiotic solutions and physisorption of antibiotic to bone graft may provide immediate protection at the surgical site, neither alternative offers lasting efficacy. Importantly, all knee revisions, infected or not, require new bone stock, commonly supplied by bone graft (estimated 30-50 cm^3 per knee) [17]–[19]. However, no current clinical technology endows this substrate with a legitimate, controlled release design for extended bioactive drug release [10], [20]–[25]. Alternatively, effort has been made to incorporate controlled local antibiotic delivery through the routine use of antibiotic-loaded bone cement (ALBC). Unfortunately, ALBC is a classic example of inadequate local antibiotic delivery controlled by a glassy, non-swelling, non-biodegradable polymer foreign body. Early burst of antibiotic is followed by extended release of drug below the therapeutic level [26]. Consequently, a new local controlled extended antibiotic delivery to treat PI is needed. The development of polymer-controlled, antibiotic bone void filler (ElutiBone™) is not only dependent on the substrate and the polymer matrix but also on the choice of antibiotic[23], [27].

Certain antibiotics, such as the aminoglycosides (e.g., gentamicin, tobramycin, etc.), are very amenable to incorporation in a polymer-controlled antibiotic bone void filler, such as ElutiBone™, due to their stability and broad spectrum activity [28]–[30]. However, the alarming rise in multidrug resistant bacteria has somewhat neutralized the bactericidal potency of the aminoglycosides [31], thereby necessitating the investigation of new microbial compounds and cocktails. Silver has a long history of use, particularly in combination with the antiseptic [32], chlorhexidine, and may prove appropriate for local PI treatment [33]. Based on the theoretical bactericidal mechanism of action, several silver salts were incorporated in ElutiBone™ BVF both with and without the addition of

Table 1. Compositional comparison of all drug-loaded BVF sample cohorts used in this release study

Cohort Name	PCL (mg)	PEG (mg)	ProOsteon 500R (mg)	Silver Salts (mg)			Chlorhexidine (mg)
				AgCl	AgNO3	Ag2CO3	
Cohort 1	150	0	350	55			
Cohort 2	135	15	350	55			
Cohort 3	112.5	37.5	350	55			
Cohort 4	150	0	350		55		
Cohort 5	135	15	350		55		
Cohort 6	112.5	37.5	350		55		
Cohort 7	150	0	350			55	
Cohort 8	135	15	350			55	
Cohort 9	112.5	37.5	350			55	
Cohort 10	150	0	350				55
Cohort 11	135	15	350				55
Cohort 12	112.5	37.5	350				55
Cohort 13	150	0	350	27.5			27.5
Cohort 14	135	15	350	27.5			27.5
Cohort 15	112.5	37.5	350	27.5			27.5

chlorhexidine to assess both the potency and synergy of drug release from this combination device in the course of this study (Table 1). Synergy is defined as an increase in the duration or efficacy of antimicrobial bioactivity when compared to either drug individually. By using a combination release strategy, ElutiBone™ has proven an effective platform for tailored, extended-duration antimicrobial release to address the complex clinical needs associated with periprosthetic orthopedic infections.

METHODS

Fabrication: ElutiBone composite BVF was fabricated as previously described[23][31]. Briefly, ProOsteon 500R (Biomet, USA) was morselized and sieved to select for particulate between 150-425μm. Polycaprolactone 10 kD (PCL, Sigma, USA) was mixed with polyethylene glycol (PEG, Sigma, USA) and then heated at 75°C to produce a molten polymer blend. The morselized ceramic, ProOsteon, and antibiotics (See table 1) were added as solids and mixed into polymer blend to form a composite matrix (n=3). The molten composite matrix was then compressed into a silicone mold (2 mm x 3 mm x 6 mm) and cooled to room temperature, forming a composite solid.

Drug Concentration Standards: Concentration standards were made in PBS for each of three silver salts (Sigma) and chlorhexidine (Sigma) from zero to 8 mg/ml (n=2). Each standard (500 μl) was used in subsequent bioactivity assays.

Antibiotic Bioactivity: Known masses of ElutiBone drug-loaded combination devices were incubated in PBS at 37°C. PBS was completely exchanged at 24h, 72h, and each week out to 12 weeks. The bioactivity of antibiotic released into the PBS milieu was determined via zone of inhibition assays (ZOIs). ZOIs were determined on BHI (Brain Heart Infusion) agar plates using 10^5 CFU of *S. aureus* according to standardized CLSI methods [35] and as previously described. *Staphylococcus aureus* (ATCC strain 49230) was cultured as previously described [23][34].

RESULTS

In this study, the antiseptic chlorhexidine and a variety of bacteriostatic silver compounds were incorporated into ElutiBone™ BVF combination devices to investigate potential synergistic antimicrobial activity against *S. aureus*, the most commonly causative organism in PI. Each agent - silver chloride, silver nitrate, and silver carbonate - was released from ElutiBone™, and their antimicrobial efficacy assessed. Silver salts are ionized to inhibit bacterial growth through interaction with cellular membrane channels, receptors and enzymes, and subsequent damage to membranes and intracellular metabolic activity [36]. No bioactivity was seen from silver carbonate release, potentially due to limited intrinsic solubility. Based on initial concentration standards, silver chloride was found to be the most effective silver salt for inhibiting growth of *S. aureus* bacterial cultures *in vitro* ($p \leq 0.05$) (see Figure 1).

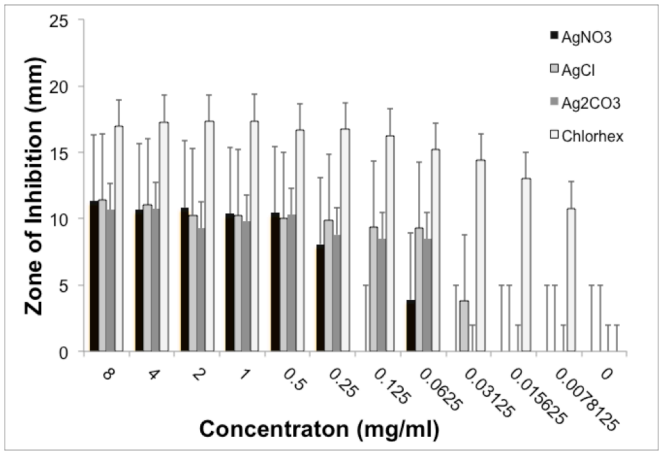

Figure 1. Spiked drug concentration standards show that silver chloride salt is the most effective silver salt based on its duration and level of efficacy from those tested.

Chlorhexidine was also released from ElutiBone™ to determine its efficacy. As a cationic bisbiguanide, chlorhexidine interacts with the cellular membrane followed by leakage of the intracellular contents[37]. Alone, chlorhexidine shows consistent inhibition for only three days (Figure 2A), indicating a rapid burst of drug from the combination BVF device.

Silver chloride was subsequently the only silver salt pursued in combination with the antiseptic chlorhexidine to assess synergistic or enhanced dual antimicrobial bioactivity against *S. aureus* beyond that seen with either individual compound. Results revealed that over the 12-week *in vitro* study, 69% of the antimicrobial assays displayed a significantly larger zone of inhibition when compared to either drug alone and seemed to be synergistic in their antimicrobial activities ($p \leq 0.05$, Figure 2), irrespective of the BVF polymer ratio, confirming the concept that ElutiBone™ BVF can be formulated to release multi-drug cocktails to produce longer-term more powerful antimicrobial effects than each drug alone. Importantly, the duration of antimicrobial efficacy was also increased with higher amounts of PCL in the polymer matrix.

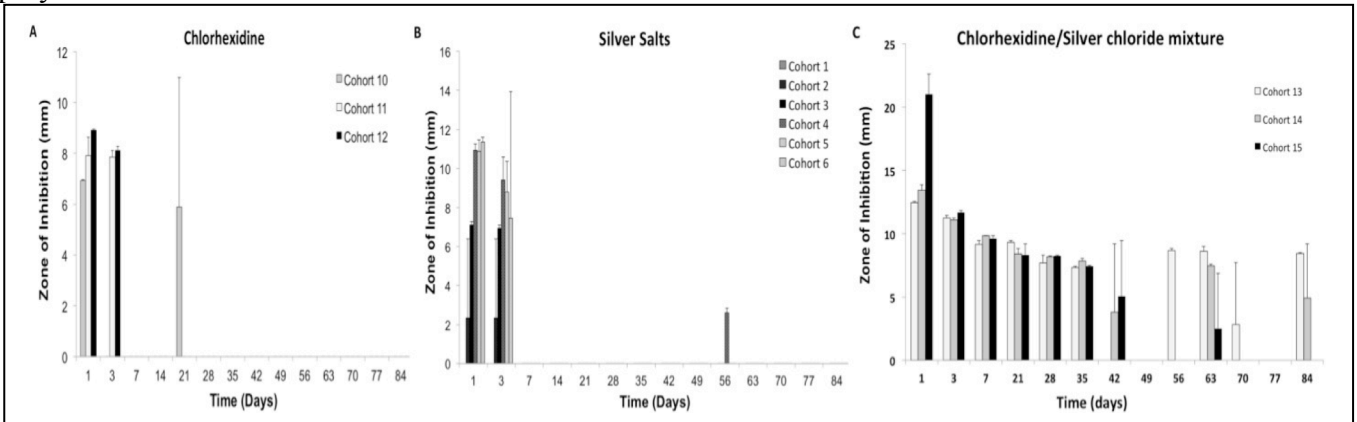

Figure 2. Combined chlorhexidine and silver chloride release from BVF formulations showing synergistic antimicrobial interactions, improving the duration of antimicrobial efficacy beyond that seen for either chlorhexidine or any of the silver salts. All BVF formulation cohorts are defined in Table 1.

DISCUSSION

Implantable local antibiotic delivery devices at orthopedic surgical sites remain among the most promising tools in infection rate reduction [27][32]. Local delivery of antibiotic mitigates resident pathogens, reduces problems of systemic toxicity, and maintains drug concentrations above the MIC at the surgical site. These properties also reduce the potential for development of antibiotic resistance [23][32][34]. Unfortunately, current standard of care for localized orthopedic drug delivery relies primarily on non-degrading, antibiotic-releasing, poly(methylmethacrylate) glassy matrices [26][38] that fail to meet the relevant regulatory and clinical criteria for use as a local antibiotic-eluting device and that create permanent foreign body implants. Thus, the problem of orthopedic infection will remain until a better and more complete solution (i.e., implant/surgery/antimicrobial protocol) is implemented.

In previous studies, we have detailed how ElutiBone™ BVF addresses many of these orthopedic infection problems [23][32][34]. To date, most of this work uses BVF designs incorporating tobramycin. Thus the scope of this study has served to further broaden the possibilities of drugs that may be used in ElutiBone™ BVF technology. Combinations of silver chloride/chlorhexidine provide an intriguing released cocktail due to the observed synergy (i.e. enhanced antimicrobial bioactivity) and extended release profile. As a single compound release, chlorhexidine was only able to provide reliable inhibition

for 3 days (Figure 2a). Chlorhexidine is known to inhibit the growth of susceptible staphylococci at an MIC <4mg/L [39] , as indirectly confirmed in Figure 1 where all standards inhibited bacterial growth. Importantly, silver chloride was determined to be the most effective silver salt, potentially due to the compound's favorable solubility and ready ionic release. The silver/chlorhexidine combination, already commercialized for antimicrobial catheter use, potentially provides additional benefit, reducing the potential for development of antimicrobial resistance [32]. Reducing the propensity for the development of multidrug resistance is critical in this system as periodic, transient pauses in bioactivity are observed (Figure 2C); however, this is not thought to be an overwhelming obstacle due to the high level of inhibition seen through 6 weeks and the intermittent strong inhibition of microbial growth to 12 weeks. Nevertheless, a thorough investigation of the release kinetics of this BVF combination device must be more thoroughly evaluated for extended durations of sub-MIC drug release.

Many other drug interactions could and should be tested in this novel system in order to broaden the scope of BVF antimicrobial efficacy. Future work will focus on *in vivo* performance and anti-infective efficacy for this combination medical device in preclinical infection models as well as detailing the mechanism of killing and extended agent release from this degradable antimicrobial BVF.

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