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An off-the-shelf plasma-based material to prevent pacemaker pocket infection

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ABSTRACT

Bacterial infection of subcutaneous "pockets" housing cardiovascular implantable electronic devices is a significant clinical complication. In this study, pacemakers encapsulated in a blood plasma-based material (PBM) composited with antibiotics were investigated for use as prophylactics against such infections. PBMs, which are made from pooled allogeneic plasma and platelets, are off-the-shelf biomaterials that can be manufactured in the form of complex 3D shapes, extrudable putties, or injectable pastes. *In vitro* studies with PBM pastes formulated with rifampicin and minocycline demonstrated antibiotic release over 6 days, activity against *Escherichia coli*, and reduced cytotoxic effects of the antibiotics on fibroblasts. The materials were also evaluated *in vivo* in a rabbit model in which pacemaker pockets were inoculated with methicillin-resistant *Staphylococcus aureus* (*S. aureus*) strain and examined 1 week later. The pockets containing the pacemaker plus *S. aureus* were grossly purulent and culture positive, whereas pockets into which PBM with antibiotics were injected around the pacemaker were free of purulence and culture negative ($p < 0.001$). None of the pockets into which PBM without antibiotics were placed demonstrated purulence, but 60% were culture positive. These results demonstrate the potential of PBMs to deliver antibiotics to diminish the incidence of pocket infections for pacemakers and other implantable devices.

1. Introduction

Bacterial infection of subcutaneous "pockets" created to house cardiac pacemakers and cardioverter defibrillators, collectively referred to as cardiovascular implantable electronic devices (CIED), is a significant problem [1]. The incidence of pocket infections ranges from 1 to 7% [2]. While the number of CIEDs procedures has been on the rise due in part to the aging population, the number of infections has increased disproportionately [3]. For instance, between 1993 and 2008, the number of CIEDs in use doubled, however, the number of infections associated with implantation of these devices more than tripled. The repercussions of infection are severe, including mortality, morbidity, and high cost of care [4], with 18% of patients with CIED infections not surviving for more than a year [5].

The most common bacterial strains involved in infections related to CIED implantations are *Staphylococcus aureus (S. aureus) and Staphylococcus epidermis (S. epidermis)* [6,7]. These bacteria can adhere to the devices and produce a biofilm, which makes the bacteria orders of magnitude more resistant to antibiotics [8–17]. Additionally, the prevalence of methicillin-resistant *S. aureus* (MRSA) is a concern in the treatment of CIED-related infections. Because CIED-related infection forms in a tissue pocket, systemically delivered antibiotics may not be effective at eliminating the infection. Biomaterials to locally deliver antibiotics may be a more effective approach, and multi-antibiotic delivery, such as a combination of minocycline and rifampicin with distinct mechanisms of action, is most likely required [18,19].

In addition to the need for localized delivery of antibiotics

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[20,21], the ideal delivery biomaterial for uses as a prophylactic against CEID infection should be: 1) easy to apply at the time of CIED implantation; 2) an enhancer of tissue pocket healing, which could reduce seroma formation and further reduce the likelihood of infection; 3) completely biodegradable so that it would not be a source for further inflammation, biofilm formation, or interfere with subsequent surgical intervention; 4) cost-effective to enable it to become a standard of care; and 5) help mitigate cytotoxicity of delivered drugs. While clinically available products address many of these requirements [6,7,22,23], they do not enhance healing, require additional attention to implant technique, are relatively expensive to manufacture, and do not possess anti-cytotoxic actions. In this study, the application of a biologically-active, blood plasma-based material (PBM) as a drug delivery vehicle that addresses all of the aforementioned requirements is demonstrated.

Plasma and platelets contain an array of growth factors and other signaling molecules that are released into an injury site upon platelet activation to initiate the healing cascade [24]. Accumulating evidence support therapies using materials derived from plasma/platelets to accelerate and enhance healing of surgical and traumatic wounds while also having an antimicrobial effect [25,26]. PBMs are novel solid to semi-solid biologically-active materials that are made from pooled allogeneic plasma and platelets [27]. PBMs are inexpensive, safe, available as off-the-shelf products with low lot-to-lot variability, shelf stable at room temperature, manufacturable in forms ranging from pliant sheets to complex 3D shapes, which can be rubbery to hard, and have tunable biomechanical and biodegradation properties. Depending on the application, additional processing can be used to turn these solid forms into extrudable putties or injectable pastes. The low-temperature manufacturing process used to make PBMs also enable drugs such an antibiotics to be added directly to these materials during processing.

To test if a PBM delivering antibiotics can effectively act as a prophylaxis against CIED infection, PBM composited with minocycline and rifampicin was formulated as a paste that could be simply delivered through a standard syringe and catheter tip into a CIED pocket and around the CIED implant. Minocycline and rifampicin were chosen because of their aggregate broad antibacterial spectrum, and based on previous reports of utility of this combination for prevention of pacemaker pocket infection [28]. PBM-incorporated antibiotic release and bioactivity was established *in vitro* and then the efficacy of the antibiotic PBM paste to inhibit infection in a rabbit pacemaker pocket infection model was demonstrated.

2. Materials and methods

2.1. PBM paste (with and without antibiotics)

PBM materials, with and without antibiotics, were supplied by Carmell Therapeutics Corp. (Pittsburgh, PA). PBMs were manufactured as previously described [27]. Briefly, plasma powder was prepared by first pooling multiple units of virally screened, frozen human plasma (with platelets) obtained from the Central Blood Bank of Pittsburgh, PA (Fig. 1). Typically, plasma pools consist of 20–50 units to ensure lot-to-lot consistency. The pooled plasma was clotted with calcium chloride, lyophilized, and ground into a powder. Two proprietary viral inactivation methods based on pasteurization and irradiation were employed to inactivate any potential viruses contained within the plasma. PBM plastics were made by mixing plasma powder and glycerol into a dough, which was then compression molded at 70 °C at 12 kpsi. For PBMs containing antibiotics, rifampicin (Sigma, St. Louis, MO) and minocycline hydrochloride (Sigma) antibiotic powders were mixed into the dough at 0.51% (on a per weight basis) prior to compression molding into solid cylindrical blocks (12 mm dia. x 6 mm height). No cross-linkers were added, which otherwise can be used to increase PBM degradation times, if needed [27]. Antibiotic levels were chosen to obtain a final concentration of 11 mg of each antibiotic per 5 ml dose of paste. Molded PBMs (with or without antibiotics) were then cryogenically milled using a Retsch CryoMill (Retsch, Newtown, PA) to create a powder. The milled PBM powder was mixed with glycerol 1:3 (by weight) to form a paste and loaded into syringes at 5 cc each. Syringes were then packaged into individual sterilization pouches and terminally gamma irradiated at 30 kGy. In principle, pastes could be directly produced in Step 5 of Fig. 1, however, that approach leaves little control over final material properties. Molding the plasma powder into a biomaterial, analogous to that seen in manufacturing a synthetic plastic, allows for more control over degradation and antibiotic release properties through controlling molding temperature, pressure, and formulation composition. Because shelf life had not been established, antibiotic and control pastes were stored at 4°C until use. PBM paste containing minocycline and rifampicin will be referred to as PBM(+MR) and PBM paste without antibiotics as PBM(-MR).

2.2. Bacterial disk diffusion assay

Antimicrobial activity of antibiotic paste was measured using a modified Kirby–Bauer disk diffusion assay [29]. Briefly, 25 mL of Difco LB broth (Becton Dickinson, Franklin Lakes, NJ) containing 1.5% (by weight) Bactoagar (Becton Dickinson) was added to 100 cm plates and stored at 4°C until use. A culture of DH5α *Escherichia coli (E. coli)* (ATCC, Manassas, VA) was grown as a stationary culture overnight at 37°C in LB Media (Becton Dickinson). The overnight culture was diluted to an OD_{600} of 0.1, 100 μL was plated, and the plate incubated at room temperature for 10 min. PBM samples (as indicated) were added directly onto the plate. Dry filter paper disks were placed on the plate and 5 μL of antibiotic solution (as indicated) was added to the disk as positive controls. The plates were then stored at 37°C overnight for 24 h. Samples were tested in triplicate.

2.3. In vitro antibiotic release

PBM plastics were made as described above, but powdered antibiotic was added into its formulation at 5% (by weight), rather than the 0.51% (by weight) used in making PBMSs to create the paste. This allowed for improved detection of the released antibiotic. PBMs were cut into 5 mm diameter disks (~1 mm thick) using biopsy punches and placed in a well plate. Disks were immersed in phosphate buffered saline (pH 7.4) at 37°C for rifampicin and 4°C for minocycline, which is temperature sensitive. Media was isolated and replaced periodically and assayed by absorbance (350 nm for minocycline/334 nm for rifampicin) to determine the concentration of antibiotic released relative to a standard curve. Additionally, minocycline release media was treated with 0.5 M EDTA for 30 min prior to measuring absorbance in order to precipitate calcium, which forms an insoluble complex with minocycline [30]. Standard curves were used to determine the amount of antibiotic released over time, and release was reported as a percentage of the initial antibiotic load within the PBM sample. Samples were tested in triplicate.

2.4. In vitro bioactivity/cytotoxicity testing

Plasma powder extract media was prepared by extracting lyophilized plasma powder (from Section 2.1) with Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Waltham, MA)