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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)

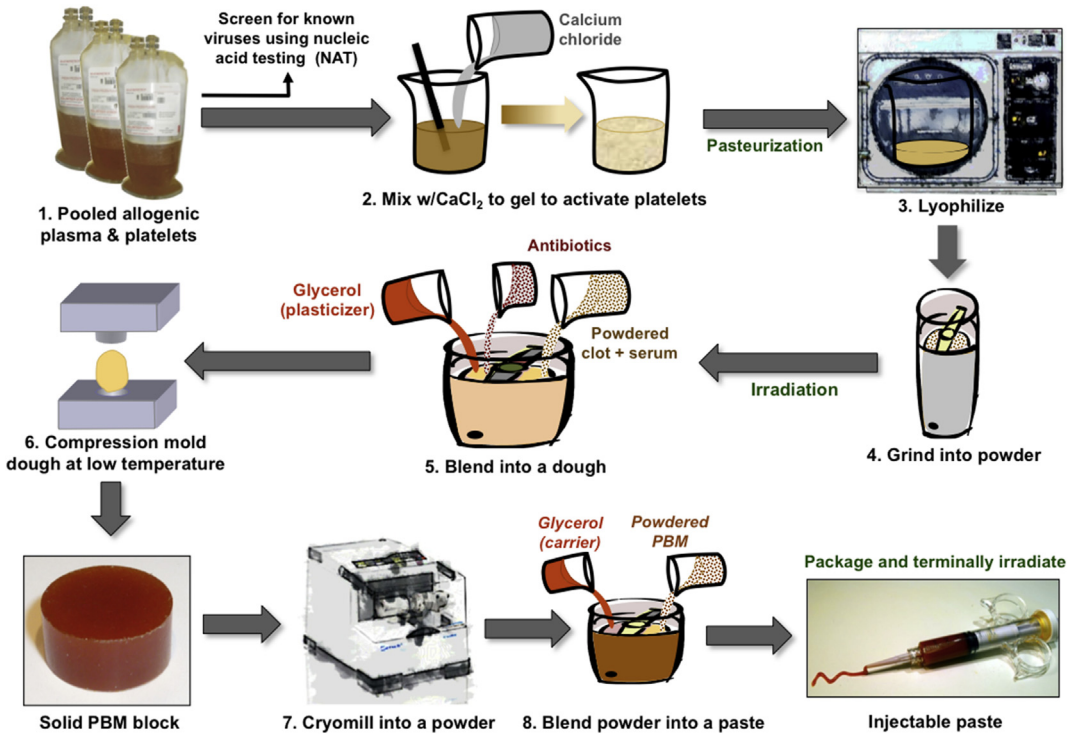

Fig. 1. Manufacture of a PBM paste with minocycline and rifampicin. The rifampicin imparts a red color to the paste.

containing 10% Gibco fetal bovine serum (Life Technologies, Grand Island, NY) and 100 μg/mL streptomycin and 100 U/mL penicillin (Life Technologies) (henceforth referred to as DMEM) on an inverting mixer for 4 h at room temperature, spinning the extract at 10,000 rpm for 10 min, and then collecting the supernatant. The supernatant was further diluted 1:2 in DMEM to make plasma powder extract media (henceforth referred to as PPEM). NIH 3T3 cells (ATCC, Manassas, VA) were seeded in DMEM at 5000 cells/well in a 96-well plate and allowed to proliferate overnight. Twenty-four hours later the media was aspirated from cells and replaced with the antibiotic (minocycline or rifampicin) treatments, which ranged from 0 to 1.0 mg/ml (0.1 mg/ml increments) in concentration and were dissolved in either DMEM or PPEM. Antibiotic concentrations ranged from 0.1 to 1.0 mg/mL, in increments of 0.1 mg/mL. Four replicate wells were tested per concentration. Overall, 3 independent experiments were performed. DMEM without antibiotic was used as a positive control. The plates were then returned to the incubator at 37°C, 5% CO_2 for an additional 24 h. Cell viability was assessed using a commercially available MTT-based assay (CellTiter 96® Proliferation Assay, Promega, Madison, WI). MTT dye was added to each well before returning the plates to the incubator for 4 h at 37°C, 5% CO_2. After 4 h, stop solution was added and to the plate, which was incubated an additional 1.5 h at room temperature, protected from light. Finally, absorbance was read at 570 nm (reference at 650 nm) and scaled to the positive control (DMEM, no antibiotic), which is defined as the Scaled Bioactivity.

2.5. In vivo evaluation

The protocol was approved by the Institutional Animal Care and Use Committee of The University of Pittsburgh and conformed to the position of the American Physiological Society on research animal use. A methicillin-resistant strain of *S. aureus* (ATCC 43300, ATCC) was inoculated onto blood agar and incubated overnight at 37°C. Fresh colonies were added to Trypticase soy broth and adjusted to equal the turbidity of a MacFarland 0.5 nephelometer standard. The concentration of the resulting suspension was adjusted by serial dilution to 5×10^5 colony forming units/cc, confirmed by plate count.

The animal model used conforms to that described by Hansen [7]. New Zealand White rabbits (2–3 kg) were anesthetized using isoflorane delivered by facemask. A broad area of the back was shaved, and the underlying skin washed with surgical scrub, wiped with alcohol, painted with betadine, and draped for surgery. Under sterile conditions, two subcutaneous symmetrical pacemaker pockets were created on either side of the spine; care was taken to ensure broad physical separation between the pockets to eliminate cross-contamination risks (Fig. 2). The dimensions of these pockets were consistent with those created in man for pacemakers, having a volume of approximately 30 mL.

Three groups of animals were studied. Pocket-sidedness was randomly assigned. In Group 1 (N = 5), Pocket #1 received pacemaker generator (Kappa™, Medtronic Inc., Minneapolis, MN) and bacterial suspension (2 mL) and Pocket #2 received pacemaker generator alone. In Group 2 (N = 7), Pocket #1 received pacemaker generator and bacterial suspension and Pocket #2 received pacemaker generator, bacterial suspension, and PBM(+MR) paste (5 mL). In Group 3 (N = 5), Pocket #1 received pacemaker generator and bacterial suspension and Pocket #2 received pacemaker generator, inoculum, and PBM(-MR) paste (5 mL). After delivery of the treatment groups into the pockets, wounds were closed using absorbable suture (Polysorb™, Covidien Inc., Manfield, MA). After full recovery, animals were returned to their cages.

Animals were then observed daily throughout a 1 week period, after which they were returned to the operating room and prepared as above. Each pocket was opened via a small incision distinct from the prior wound, through which two sterile cotton swabs were inserted, sequentially. Swabs were manipulated vigorously

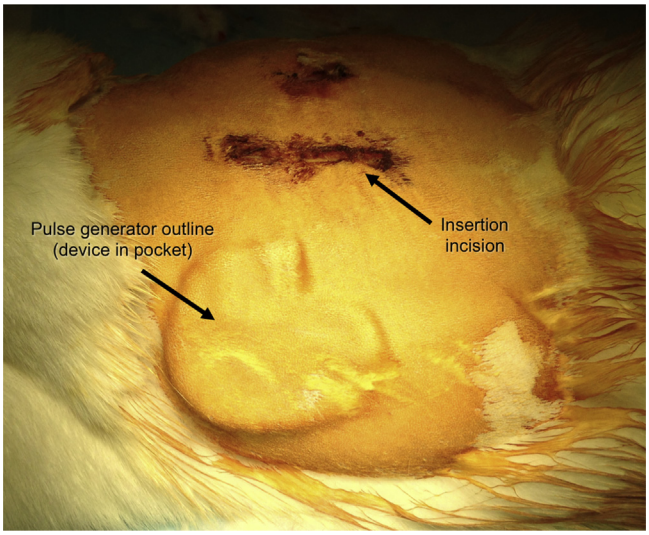

Fig. 2. Photograph of representative pacemaker pocket, shown closed with entry wound and device *in situ*.

throughout the pocket, with care taken to sample all device surfaces. Animals were then euthanized, and the incision extended. The pacemaker was removed under sterile conditions, and swabbed on front and back surfaces, as well as in and about the lead ports. The device was then placed in a nonionic surfactant and emulsifier solution (Tween® 80, Sigma–Aldrich) and subjected to vortex and sonication. A closed container containing the device immersed in 100 mL of solution was vigorously vortexed for 30 s, followed by sonication for 5 min. Cotton swabs of the sonicant solution were streaked onto trypticase soy agar plates, each of which was incubated at 37°C. Plates were examined daily for the presence of bacterial colonies. The finding of one or more colonies on any plate at or before 72 h was defined as a positive pocket culture result, and the absence of colonies defined as a negative culture result. Recovered colonies were confirmed as *S. aureus* using Gram stain and coagulase testing (Staphaurex™, Remel Inc., Lenexa, KS). After obtaining culture samples, each pocket was excised en bloc, and transmural sections from representative areas taken. Specimens were fixed in 10% formalin and embedded in paraffin blocks. Tissue sections, 5 μm thick, were mounted onto slides, which were stained using Hematoxylin and Eosin.

2.6. Statistical analysis

Data are reported as mean ± standard deviation, unless otherwise stated. Categorical variables were assayed using Fisher Exact Test. Cytotoxicity data were analyzed using ANOVA followed by a protected LSD post-hoc test. For each statistical test, a p-value of <0.05 was considered significant.

3. Results

3.1. Bacterial disk diffusion analysis

The manufacture of PBMs utilizes moderate temperatures (70°C), which could potentially inactivate incorporated antibiotics. Therefore, bacterial disk diffusion assays were used to determine if there was any remaining antibiotic activity after being incorporated into the PBM and PBM paste. Both minocycline and rifampicin released from the PBMs were active indicating that both the PBM manufacturing process did not destroy the antibiotics and that the antibiotics were freely released (Fig. 3).

3.2. In vitro antibiotics release kinetics

Free release of antibiotics from PBMs was determined *in vitro* by using absorbance to detect the natural chromophores of the antibiotics (Fig. 4). Rifampicin was primarily released within the first 24–48 h, with approximately 95% of antibiotic released within 6 days. Similarly, most of the minocycline was released in the first 24–48 h; however, only approximately 60% of the total minocycline was released over 6 days. This lower level of release could possibly result from the minocycline being bound into the matrix of the PBM. The PBMs were then degraded in trypsin to see if any additional antibiotic could be recovered, resulting in less than 5% additional recovery (data not shown), and indicating that the antibiotics were not entrapped within the PBM.

3.3. In vitro bioactivity and cytotoxicity

NIH 3T3 fibroblasts were incubated with antibiotics in the presence of PPEM. Cytotoxicity was measured using a commercial MTT metabolic assay. Cells treated with rifampicin in PPEM exhibited reduced antibiotic cytotoxicity relative to cells treated with rifampicin in DMEM (Fig. 5A), meaning that the plasma powder extract provided the cells a protective effect against the antibiotic. PPEM led to reduced cytotoxicity at all rifampicin concentrations ($p < 0.05$) except 0.8, 0.9, and 1.0 mg/mL where p-values were 0.11, 0.12, and 0.22, respectively. Overall, minocycline was not as cytotoxic to NIH 3T3 cells; however, cells treated with minocycline in PPEM exhibited increased bioactivity (i.e. increased cell viability) relative to cells treated with minocycline in DMEM alone ($p < 0.05$) (Fig. 5B).

3.4. In vivo model

In Groups 1, 2, and 3, all pockets containing the pacemaker generator and bacterial suspension ($n = 5$, 7, and 5, respectively)

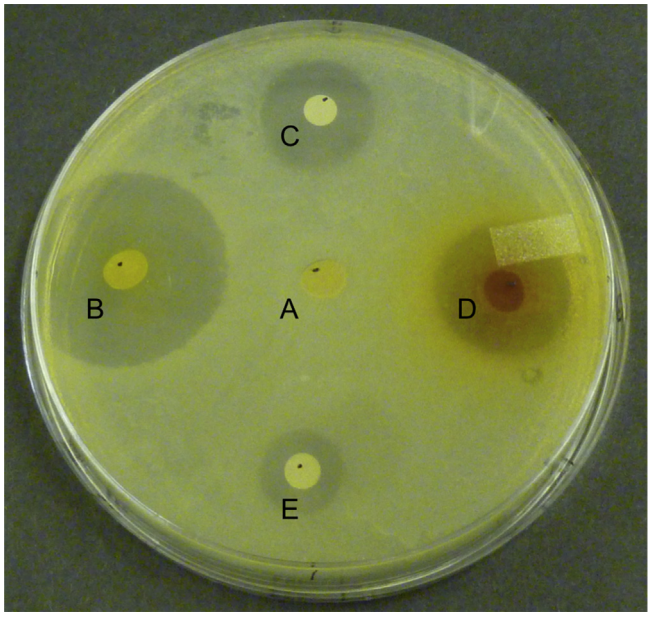

Fig. 3. PBM-delivered antibiotics demonstrate bioactivity. PBMs were tested in a modified disk diffusion assay utilizing *E. coli*. Samples include: (A) PBM without antibiotics; (B) PBM containing 5% (by weight) minocycline; (C) Filter paper infused with 10 mg minocycline; (D) PBM containing 5% (by weight) rifampicin; (E) Filter paper infused with 10 mg minocycline. Note that PBM without antibiotics demonstrates no activity against *E. coli*.

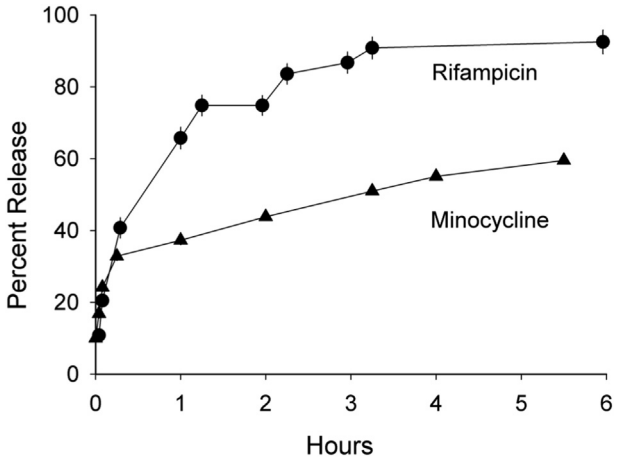

Fig. 4. *In vitro* antibiotic release from PBMs. Minocycline (△) and rifampicin (○) were individually formulated into PBM disks. The disks were incubated in PBS and the released antibiotics were quantified by absorption spectrophotometry. Symbols represent the mean of 3 independent experiments.

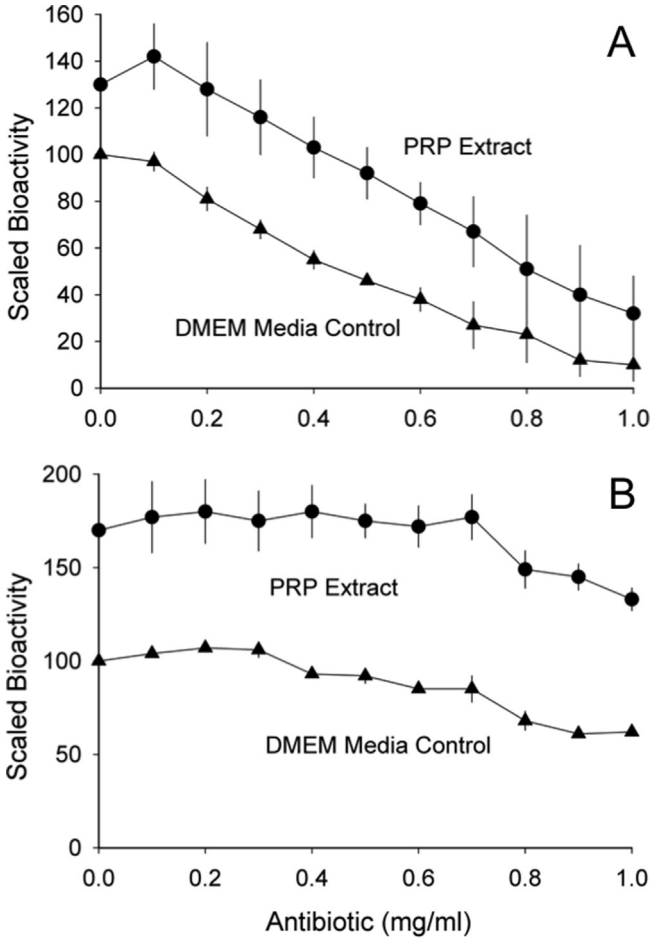

Fig. 5. PBM anti-cytotoxic effects on NIH 3T3 cells. Cells were incubated in DMEM media with (▲) or without (●) PBM plasma powder extract containing the indicated antibiotic concentration for rifampicin (A) or minocycline (B). Cytotoxicity was measured using a standard MTT metabolic assay. Symbols represent the mean of 3 independent experiments.

were culture positive for *S. aureus* and demonstrated gross purulence (Fig. 6). In Group 1, all pockets containing the pacemaker pulse generator alone (n = 5) were culture negative. In Group 2, all pockets containing the pacemaker generator, bacterial suspension, and PBM(+MR) paste (n = 7) demonstrated no purulence and were culture negative. PBM was observed to aggregate around the device and encapsulate it (Fig. 6), and adjacent tissue appeared healthy (Fig. 7). In Group 3, among pockets containing the pacemaker generator, bacterial suspension, and PBM(-MR) paste (n = 5), none demonstrated purulence, but three were culture positive for *S. aureus*. Compared to pockets containing the device and bacterial suspension alone treatment, PBM(+MR) paste led to statistically lower culture positives ($p < 0.001$) as did treatment with PBM(-MR) paste ($p < 0.05$).

4. Discussion

Bacterial infection of subcutaneous pockets housing CIEDs is a significant clinical complication, due to associated mortality, morbidity, misery, and high cost [1]. The TYRX™/AIGISRx Antibacterial Envelope (Medtronic CRDM/TYRX, Monmouth Junction, NJ) is the only currently approved treatment modality for infection prevention. This device is a knitted envelope consisting of either non-absorbable (AIGISRx) or absorbable filaments (AIGISRx R) coated with an absorbable layer of polyarylate containing rifampin and minocycline. Pacemaker or defibrillator pulse generators are placed into envelopes, which are then inserted into the subcutaneous pocket. Antibiotic elution within the pocket proceeds over a 7–10 day period.

As an alternative modality for pocket infection prevention, we have engineered a PBM delivery vehicle composited with antibiotics. Antibiotic types, quantity, and delivery kinetics for PBM are similar to AIGISRx and AIGISRx R. However, we believe that the PBM vehicle has a number of advantages. First, full PBM absorption is expected to be significantly faster that for the envelope. Combined with the intrinsic properties of PBM, this is expected to engender significantly less fibrosis than with the inert envelope material. Second, the AIGISRx and AIGISRx R envelopes are constructed in a medium and large size in order to accommodate all CIEDs, which vary in shape significantly among manufacturers. This often leaves a considerable space between the envelope surface and the pulse generator surface. In addition, as the envelope is considerably larger than the pulse generator, significant additional dissection of the pocket is needed to accommodate, which poses risk for bleeding. As we demonstrate, the paste consistency of PBM allows it to form a custom, closely adherent “envelope” around the pulse generator. No additional tissue dissection is required. Finally, unlike the inert envelope material, PBM is expected to actively promote healing of the pocket and mitigate against cytotoxicity of the locally delivered rifampin and minocycline. As a result of a relatively rapid absorption time (PBM expected ~4 weeks), cytoprotective and wound healing properties, PBM paste may further mitigate infection, in both the short and long terms, leading to less time to wound healing and stabilization of the implanted CIED within muscle; however, additional studies will be required to assess the importance of these attributes.

To assess the ability to maintain antibiotic activity when composited with PBMs, bacterial disk diffusion assays demonstrated that both rifampicin and minocycline can be incorporated into a PBM formulation, survive PBM manufacturing conditions, and be released in an active form. While it was demonstrated that the terminally sterilized antimicrobial paste retained its activity post-irradiation and had a shelf life of at least 30 days at 4°C (data not shown), it is anticipated that the actual shelf life is at least 6–12 months at room temperature, but that remains to be tested.

Assessment of antibiotic release kinetics *in vitro* demonstrated that the majority of minocycline or rifampicin is freely released within the first 72 h, with approximately 100% released within 1

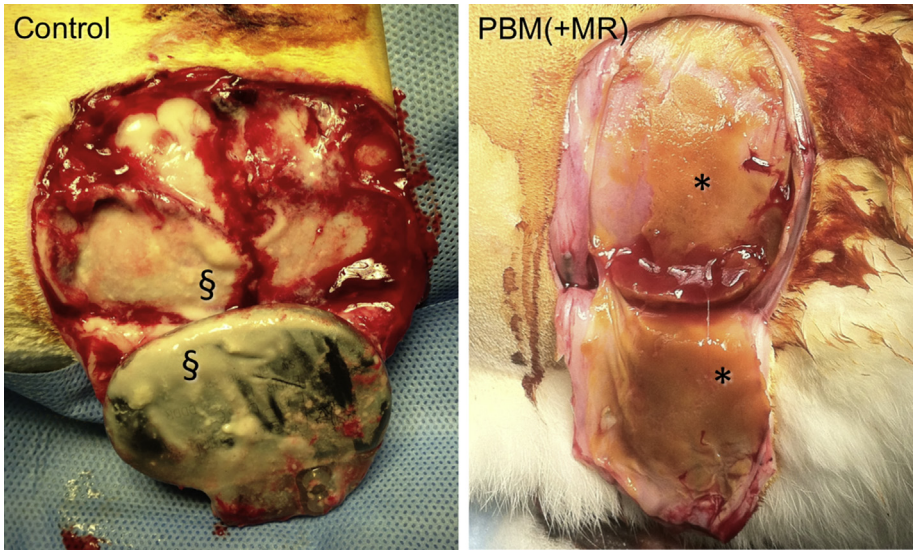

Fig. 6. Photographs of pacemaker pockets, opened at 1 week. The image on the left (control) is representative of a pocket which included pacemaker (arrow) and bacterial inoculum. Exuberant purulent material is observed along the wall of the pocket and the surface of the device (§). The images on the right are representative of a pocket which included pacemaker, bacterial inoculum, and PBM(+MR) material (*). The PBM material is seen to encapsulate the pacemaker (inset), and coat the walls of the pocket contiguous to the pacemaker. There is no sign of purulence.

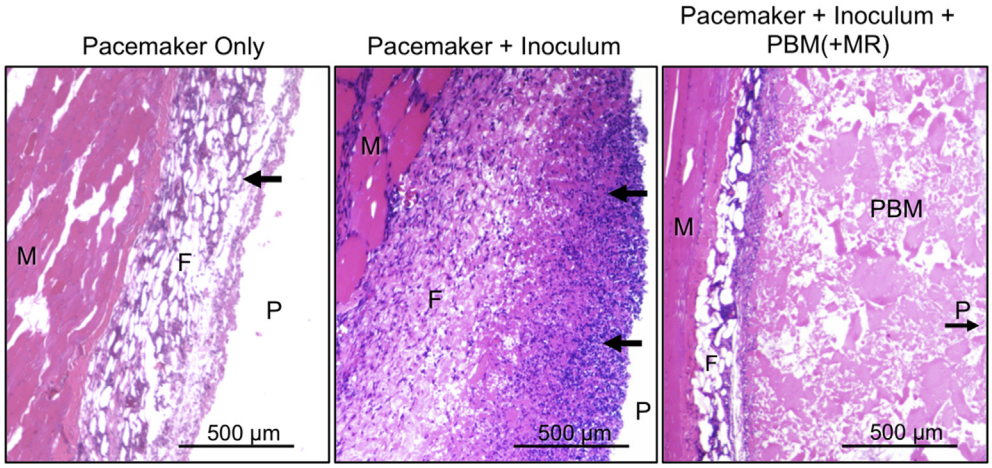

Fig. 7. Photomicrographs demonstrating differences in pockets containing pacemaker alone (left panel), pacemaker + bacterial inoculum (center panel), and pacemaker + inoculums + PBM(+MR) material (right panel). In each picture, P represents the space occupied by the pacemaker pulse generator, M the muscle layer from the chest wall comprising the pocket "floor," and F the fascial layer which was in direct contact with the pacemaker. In the pacemaker alone pocket (left panel), the fascial layer is covered with a thin, sterile inflammatory exudate (arrow). In the pocket with bacterial inoculum added (center panel), a thick exudate is apparent, which is rife with bacteria (arrows). In the pacemaker-PBM pocket with bacterial inoculum (right panel), the fascial plane is coated with the PBM material and no bacteria were observed.

week. Although the rifampicin was almost fully recovered, recovery of minocycline was only about 60%. Minocycline is heat sensitive and it is possible that some minocycline is lost during the PBM compression molding at 70°C. Additionally, minocycline is known to bind plasma proteins, particularly albumin [31], which may interfere with its detection.

Many locally administered antibiotics are associated with cytotoxicity. It has been reported that platelet-rich plasma (PRP) preparations can reverse the cytotoxic effects of corticosteroids [32–34], antibiotics [33], and anesthetics [32,34]. Therefore, the ability of the plasma powder component of the PBM to be anti-cytotoxic was examined *in vitro* for NIH 3T3 fibroblasts as a model cell line. While rifampicin demonstrated significantly more cytotoxicity towards NIH 3T3 cells than minocycline, these studies demonstrated the cytotoxicity of the antibiotics was significantly reduced when using extracts from the plasma powder portion of the PBM formulation. This significantly improved the metabolic activity of the cells, which was a result of the growth and regenerative factors released into the media, as described previously [27]. This cytoprotective effect would need to be confirmed in an *in vivo* setting, but this may indicate that PBM-based delivery systems would allow for higher antibiotic concentrations to be utilized or possibly allow for the use of more potent but cytotoxic antibiotics.

The materials were also evaluated *in vivo* in a rabbit model in which pacemaker pockets were inoculated with methicillin-resistant *S. 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 with similar antimicrobial effects as reported for TYRX [7,22].

Materials derived from blood plasma have been the subject of increasing interest for a variety of clinical applications [25–27]. In distinction to materials derived from platelet concentrates, PBM was derived from plasma in its entirety. This may prove important, given that a number of plasma proteins with possible roles in infection prevention that have been described [26]. In this regard, it is of interest that none of the pockets treated with PBM(-MR) demonstrated purulence, and 2 of 5 were culture negative. Given that the bacterial load that precedes infection in typical clinical practice is far smaller than that tested in the present study, it is conceivable that PBM(-MR) could be clinically effective. Antimicrobial effects of PBM could be attributed to immunological cell recruitment.

Despite best practice, it is likely that during some, if not all, pacemaker implantation procedures, bacterial contamination of the pocket occurs. Subsequent emergence of overt infection is related to a variety of factors, including bacterial species, virulence, load and location, peculiarities of the tissues comprising the pocket, and systemic competency in preventing bacterial purchase. Once manifest, infection generally cannot be cleared without removal of all pacemaker system hardware, due in part to formation of biofilms secreted by microbes onto device surfaces [35]. Preventing this sequence with a prophylactic PBM and antibiotic strategy creates a bactericidal pocket environment with minimal risk of systemic toxicity.

In addition to its efficacy, the low cost of manufacture of PBMs is another significant advantage of this novel technology. It is estimated that 5 cc of PBM containing minocycline and rifampicin could be manufactured, packaged, and terminally sterilized for under $20 per syringe. This would allow for wide utilization of this product in the U.S. as well as in the developing world, where infections are of increasing concern but price pressures represent a significant barrier to the introduction of novel therapeutic technologies.

We note several limitations to these data. First, only *S. aureus* was utilized. For the purpose of this experiment, we sought a species that represented the bulk of clinical infections, grew aggressively, and formed biofilms on prosthetic surfaces [36]. It is possible that the efficacy of PBM(+MR) paste demonstrated in the present study would not have been observed using other organisms also encountered in clinical practice. However, minocycline and rifampicin alone provide broad-spectrum coverage for implanted devices [20,21,28]. Second, the study was limited to pacemaker pulse generators, which have smaller surface areas relative to other implantable electrical devices, including defibrillators, neural stimulators, and drug infusion systems. The generators were also not attached to intravascular leads. It is conceivable that such differences would have exceeded the ability of the PBM strategy as currently configured. Third, infection prevention efficacy was evaluated 1 week after implantation. Although it is reasonable to expect that a bactericidal pocket environment created in the first few days after surgery will reduce the clinical burden, this remains to be demonstrated. Fourth, pocket healing was not specifically studied, and so no conclusions can be drawn as to whether PBM altered the pace of this process. Finally, more pre-clinical and clinical data will be necessary to understand the impact of locally delivered, high dose, broad-spectrum antibiotic therapy on the emergence of bacterial resistance and on the health of tissues comprising the pocket region.

5. Conclusions

PBMs are biomaterials made from blood plasma/platelets and designed to stimulate the repair of damaged tissues. In this work we have shown that PBMs can also effectively deliver active antibiotics. *In vitro* PBM-delivered minocycline and rifampicin eliminated *E. coli* in disk diffusion assays. *In vivo,* a PBM paste delivered minocycline and rifampicin to eliminate methicillin-resistant *S. aureus* in a rabbit pacemaker pocket infection model. The PBM paste alone demonstrated some effectiveness in eliminating *S. aureus* in 2 of 5 infected pockets, possibly due to the PBM stimulating the body's immune system. Although these studies demonstrate proof-of-concept and support the hypothesis that a PBM system to deliver antibiotics can act as a prophylaxis against CIED infection, more work will be needed to fully characterize antibiotic release and activity, optimize the PBM formulation, and understand its ability to eliminate infection and heal the pacemaker pocket.

Conflicts of interest

Carmell Therapeutics Corporation had no input into the rabbit pacemaker pocket study design, data acquisition, or data analysis.

Drs. Schwartzman, Weiss and Campbell are named as inventors on a provisional patent application, to be assigned to Carnegie Mellon University and the University of Pittsburgh, which covers PBM for the field of use of implanted electronic medical device infection prevention.

Dr. Schwartzman is a consultant for the following companies: Atricure, Avery–Dennison, Biosense, QuantMD, and Medtronic. He receives research grant support from the following companies: Biosense, Boston Scientific, and Medtronic.

Dr. Pasculle has no disclosures.

Ms. Ceceris was an employee of Carmell Therapeutics Corporation during the performance of the study.

Dr. Smith is an employee of Carmell Therapeutics Corporation.

Prof. Weiss is a co-founder of Carmell Therapeutics Corporation and holds equity in the company.

Prof. Campbell is a co-founder of Carmell Therapeutics Corporation and holds equity in the company.

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