Efficacy of a Vancomycin/Tobramycin-doped Polyvinyl Alcohol (PVA)-Polymeric Dicalcium Phosphate Dehydrate (P-DCPD) Composite for Prevention of Periprosthetic Infection in a Mouse Pouch Infection Model Implanted with 3D-printed Porous Titanium Cylinders

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INTRODUCTION:

Prosthetic joint infection (PJI) is a challenging problem. Irrigation, intravenous antibiotics, and sterile technique are usually used for prevention; however, in the presence of bacterial infection and/or biofilms these measures are generally not effective. Other strategies to prevent infection involve modification of the physiochemical properties of the implants themselves to achieve potential bactericidal effects, bacterial anti-adhesion, and enhanced osteointegration. This study evaluated the effect of antibiotic doped polyvinyl alcohol (PVA)-polymeric dicalcium phosphate dehydrate (P-DCPD) composite on prevention of PJI in a mouse pouch infection model implanted with 3D printed porous titanium (Ti) cylinders (400 µm pore size).

METHODS:

Air pouches were created in 30 female BalBc mice (n=10 per group). A 0.5-1 cm incision was then made over the pouch area, and the pouch membrane was cut to open the pouch for the placement of the Ti cylinders (Figure 1). Pouches were implanted with either porous Ti cylinders only (negative control), Ti cylinders and Staphylococcus aureus (S. aureus) ($1X10^6$ colony forming units (cfu)) (positive control), or Ti cylinders preloaded with antibiotic-loaded PVA-P-DCPD and S. aureus ($1X10^6$ cfu) (treatment group). Mice were sacrificed 28 days after implantation. After sacrificing the mice, pouch tissues were harvested and fixed in 10% buffered neutral formalin (BNF) and processed for histology by creating 5 µm sections stained with Hematoxylin and Eosin (H&E) (Figure 2). Additionally, pouches were washed with 3 ml of sterile saline, and the washout was collected for quantitative bacterial analysis. The cylinders were also collected and sonicated for bacterial analysis.

RESULTS:

There were no detectable bacteria in the washings or in the Ti cylinder sonicate (0 cfu/ml) following implantation with antibiotic doped PVA-P-DCPD or in the negative controls. Bacteria were present in the washings of the positive control (1,894 ± 2,455 cfu/ml) and was significant compared to the treatment group (0 cfu/ml) ($P<0.001$) (Table 1). Similarly, sonicates from the Ti discs had significantly less bacteria in the treatment group compared to positive controls (0 cfu/ml vs 22,233 ± 33,735 cfu/ml, $P=0.002$) (Table 1). Histological examination showed varying degrees of inflammation in the pouch tissues. The tissues from the treatment group showed minimal inflammation and no signs of bacterial colonies or biofilm formation. Tissues from the positive control group exhibited moderate to severe inflammation, with visible bacterial colonies and biofilm formation. The negative control group, as expected, displayed normal tissue morphology with mild inflammation due to the presence of the implant.

DISCUSSION AND CONCLUSION:

PJI remains problematic and research into its treatment is of great importance. While porous Ti implants are used in TJA for their osseointegrative properties, the porous surface also provides a surface for bacterial ingrowth and ongrowth and resultant infection. Pre-treatment of these implants with Vancomycin and Tobramycin doped PVA-P-DCPD led to a significantly reduced bacterial load in porous coated Ti implants and effectively eradicated *S. Aureus* infection (0 cfu/ml) in this mouse pouch model. The same VAN/TOB-PVA-P-DCPD composite used in this study was shown to be effective at decreasing bacterial load in a rat knee *S. Aureus* infection model with implanted porous Ti discs. Additionally, the VAN/TOB-PVA-P-DCPD composite used in our study is non-toxic to surrounding tissues with no inferior impact on osteoblastic cells. Although the structure and physiochemical makeup of VAN/TOB-PVA-P-DCPD composite is less studied than some other coatings, P-DCPD is natural in composition (calcium and phosphate), has adequate mechanical strength, and has the ability to distribute antibiotics with anti-washout properties. The results of our current study further confirm that a pre-coated antibiotic loaded PVA-P-DCPD composite is efficacious at decreasing bacterial burden on infected orthopaedic implants and may also be used to prevent the occurrence of infection.

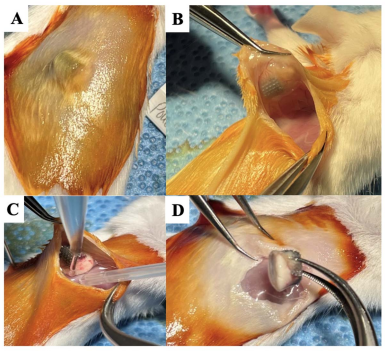

Figure 1: Gross images of surgical steps of the pouch cavity with saline wash and implant harvest 28 days after Ti cylinder implantation and bacterial inoculation. (A) Air pouch cavity with Ti cylinder visible inside. (B) Pouch cavity open with Ti cylinder visible inside. (C) Pouch cavity washout with saline and collection of washing. (D) Harvest of the Ti cylinder.

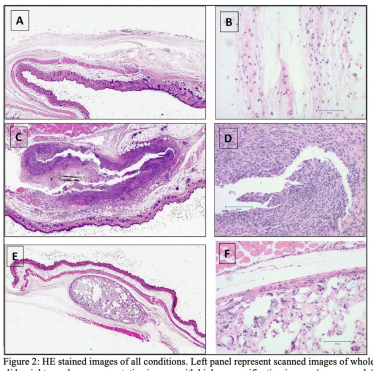

Figure 2: HE stained images of all conditions. Left panel represent scanned images of whole slide, right panel are representative images with higher magnification images (same sample). A-B for Group 1(negative control), C-D for Group 2 (positive control) and E- F for the treatment group (PDCDD with antibiotics).

Table 1. Turkey HSD Post Hoc test. Mean bacterial amount with 95% confidence intervals in parentheses below group name. Significance set at P<0.05.

WASH		
Group	**Group**	**p-value**
Ti cylinder only (0 cfu/ml)	Bacteria + Ti cylinder (1894 ± 2455 cfu/ml)	**<0.001**
Ti cylinder only (0 cfu/ml)	Bacteria + Ti cylinder + antibiotic doped P-DCPD (0 cfu/ml)	1.000
Bacteria + Ti cylinder (1894 ± 2455 cfu/ml)	Bacteria + Ti cylinder + antibiotic doped P-DCPD (0 cfu/ml)	**<0.001**
CUMULATIVE SONICATE		
Group	**Group**	**p-value**
Ti cylinder only (0 cfu/ml)	Bacteria + Ti cylinder (22233 ± 33735 cfu/ml)	**0.002**
Ti cylinder only (0 cfu/ml)	Bacteria + Ti cylinder + antibiotic doped P-DCPD (0 cfu/ml)	1.000
Bacteria + Ti cylinder (22233 ± 33735 cfu/ml)	Bacteria + Ti cylinder + antibiotic doped P-DCPD (0 cfu/ml)	**0.002**