Efficacy of a saline wash plus vancomycin/tobramycin-doped PVA composite (PVA-VAN/TOB-P) in a mouse pouch infection model implanted with 3D-printed porous titanium cylinders

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Aims

The efficacy of saline irrigation for treatment of implant-associated infections is limited in the presence of porous metallic implants. This study evaluated the therapeutic efficacy of antibiotic doped bioceramic (vancomycin/tobramycin-doped polyvinyl alcohol composite (PVA-VAN/TOB-P)) after saline wash in a mouse infection model implanted with titanium cylinders.

Methods

Air pouches created in female BalBc mice by subcutaneous injection of air. In the first of two independent studies, pouches were implanted with titanium cylinders (400, 700, and 100 µm pore sizes) and inoculated with *Staphylococcus aureus* (1×10^3 or 1×10^6 colony-forming units (CFU)/pouch) to establish infection and biofilm formation. Mice were killed after one week for microbiological analysis. In the second study, pouches were implanted with 400 µm titanium cylinders and inoculated with *S. aureus* (1×10^3 or 1×10^6 CFU/pouch). Four groups were tested: 1) no bacteria; 2) bacteria without saline wash; 3) saline wash only; and 4) saline wash plus PVA-VAN/TOB-P. After seven days, the pouches were opened and washed with saline alone, or had an additional injection of PVA-VAN/TOB-P. Mice were killed 14 days after pouch wash.

Results

The first part of the study showed that low-grade infection was more significant in 400 µm cylinders than cylinders with larger pore sizes ($p < 0.05$). The second part of the study showed that saline wash alone was ineffective in eradicating both low- and high-grade infections. Saline plus PVA-VAN/TOB-P eradicated the titanium cylinder-associated infections, as manifested by negative cultures of the washouts and supported by scanning electron microscopy and histology.

Conclusion

Porous titanium cylinders were vulnerable to bacterial infection and biofilm formation that could not be treated by saline irrigation alone. Application of PVA-VAN/TOB-P directly into the surgical site alone or after saline wash represents a feasible approach for prevention and/or treatment of porous implant-related infections.

Bone & Joint Research

Article focus

- The treatment efficacy of an antibiotic-loaded bioceramic, polyvinyl alcohol polymeric dicalcium phosphate dehydrate composite (PVA + PDCPD) on an infection around a porous titanium implant was studied in a mouse pouch model.

Key messages

- Periprosthetic infection harboured by the presence of a porous metal implant can be prevented or treated using a resorbable bioceramic with biphasic release of loaded vancomycin and tobramycin.

Strengths and limitations

- The strength of this study was the use of a simple and repeatable animal model to investigate the therapeutic efficacy of an antibiotic-loaded resorbable bioceramic composite (PVA-VAN/TOB-PDCPD) for treatment of a *Staphylococcus aureus* infection around a porous titanium implant.
- The study was limited by a lack of quantitative measures of the biofilm mass and the descriptive nature of the SEM.
- In addition, only one strain of bacteria was used for testing, which may limit extrapolation to other forms of infection.
- Other limitations of the study are use of only female mice and lack of animal randomization.
- Blinding to treatment was difficult since the surgeries were performed by a single surgeon, and the tested material is physically different from the control.

Introduction

Implant-associated infection is one of the leading causes for revision after total joint replacement (TJR).[1] Saline irrigation is frequently used in TJR to reduce the risk of or to treat infection, but its antibacterial effect is very limited in the presence of contaminated medical devices.[2,3] A commonly used treatment option for infected TJR is the debridement, antibiotics, and implant retention (DAIR) procedure,[4] which is considered a successful procedure but remains associated with high failure rates, especially in *Staphylococcus* infections.[5]

Manufacturing of porous titanium implants by 3D printing is now common for TJR.[6] The porosity and pore size of the titanium implants have a significant impact on bone ingrowth and osseointegration.[7-9]

However, there is a clinical concern that the interconnected pore structures of these implants may provide a niche for bacterial adhesion and growth.[10] In addition, it is unclear whether changes in surface topography created by remnant partially melted Ti6Al4V particles during 3D printing influence the behaviour of bacteria and the host's cells.[11] Xie et al[12] reported that the porous structure and rough surface of 3D printed titanium scaffolds were more favourable for bacterial adhesion and growth compared to implants with a smooth surface in vitro. More studies are needed to determine whether a 3D-printed porous titanium implant increases the risk of implant-related infection in vivo, and whether the pore sizes play a role in bacterial adherence and growth.

A clinically relevant animal model for porous implant-associated infections is essential for evaluating the efficacy of bactericidal biomaterials in vivo.[13] Current animal models can be initiated by inoculation of a bacterial suspension at the site of implantation,[14,15] the use of a pre-colonized implant,[16] or a mixed model using both a pre-colonized implant and a bacterial suspension.[17] For the work herein, bacterial inoculation at the implantation site was initiated with a known number of bacteria to imitate the clinical settings of low-grade and high-grade infection status in vivo. Some authors have previously developed and applied a mouse pouch model for device-associated infection,[18] and found that a saline wash, although partially effective, could not eliminate a wound infection in the presence of contaminated sutures, especially sutures with rough surface[19] and/or larger contact surfaces.[2] More efforts are needed to develop antibiotic-eluting biomaterials that are biocompatible, biodegradable, and capable of delivering antibiotics in a controlled and sustained manner.

Injectable dicalcium phosphate dihydrate (brushite or dicyclopentadiene (DCPD)) ceramics have not been historically preferred for the local delivery of antibiotics due to limitations such as poor anti-washout and burst drug release.[20] Ren et al[21] developed a new injectable polyphosphate DCPD ceramic (P-DCPD) via a setting reaction of calcium polyphosphate (CPP) hydrogel with tetracalcium phosphate (TTCP). P-DCPD has significant advantages over the original DCPD, including better mechanical strength, excellent anti-washout, and sustained drug release.[22] Vancomycin (VAN) and tobramycin (TOB) are frequently used together in clinical practice,[23] and provide good coverage for a high proportion of both Gram-positive and Gram-negative pathogens that are frequently encountered in implant-related infections.[24] We recently reported on the release profile of VAN and TOB-doped P-DCPD (VAN/TOB-P) and found that > 90% of VAN was released within three days, followed by a slow and sustained release over 28 days.[22] The early burst release of antibiotics at high concentration right after the ceramic implantation is clinically desirable to inhibit bacterial growth at the site of surgical infections. The sustained antibiotic release thereafter is expected to prevent the growth of residual bacteria and biofilm formation. Thus, the biphasic model of early burst VAN release with a sustained release of TOB from VAN/TOB-P may be of a particular clinical benefit. Polyvinyl alcohol (PVA) is a water-soluble polymer. PVA hydrogel represents a promising fast degrading matrix for drug release because of its biocompatibility and proven mechanical strength.[25] Therefore, a combination PVA-VAN/TOB-P composite paste was developed by embedding VAN/TOB-doped P-DCPD powders in PVA gel matrix capable of delivering antibiotics in a biphasic and sustained pattern.

The purpose of this study was to: 1) develop and optimize a clinically relevant mouse pouch model for both low-grade infection (inoculation of *S. aureus* at 1×10^3 CFU per pouch) and high-grade infection (inoculation of *S. aureus* at 1×10^6 CFU per pouch) in the presence of porous titanium cylinders with defined pore sizes; and 2) evaluate the therapeutic efficacy of saline wash plus PVA-VAN/TOB-P composite paste in a mouse pouch model of porous titanium cylinder implant-associated infection.

Table I. Mice groups for Study 1.

Group	N	Titanium cylinders	Xen29 1 × 10^3	Xen29 1 × 10^6	Description
I	6	400 µm	n = 3	n = 3	XN29 bacteria were injected into the pouch after titanium cylinder implantation. The mice were killed 7 days after implantation. Pouch washout, pouch tissue, and implanted titanium cylinders were collected for testing.
II	6	700 µm	n = 3	n = 3	
III	6	1,000 µm	n = 3	n = 3	

Table II. Mice groups for Study 2.

Group	Description	Xen29 1 × 10^3	Xen29 1 × 10^6
I	Control pouch (no Xen29)	n = 6	n = 6
II	Xen29 + Ti cylinder, no wash	n = 6	n = 6
III	Xen29 + Ti cylinder, wash only	n = 6	n = 6
IV	Xen29 + Ti cylinder, wash + PVA-VAN/TOB-P	n = 6	n = 6

Methods

Materials

All chemicals used for this study were analytical-grade and purchased from Sigma-Aldrich (USA) if not stated otherwise. Kanamycin-resistant *S. aureus* strain (Xen29) was obtained from Caliper Life Science (USA). Vancomycin was obtained from NDC Technologies (USA) and Tobramycin from X Gen Pharmaceuticals (USA). Female BALB/cJ mice (12 weeks old with a mean weight of 20 g) were purchased from Charles River Laboratories (USA).

Preparation of antibiotic doped PVA/ceramic composite paste

Preparation of VAN/TOB doped PVA/ceramic composite paste was described in detail in our previous publication.[26] Briefly, the powder form of P-DCPD doped with the antibiotics of choice (VAN or VAN/TOB) was prepared and then mixed with PVA (molecular weight approx. 205,000 Daltons) gel to produce a paste that can be injectable, as shown in Figure 1.[26]

Preparation of sterilized titanium cylinders for animal testing

3D-printed porous titanium cylinders (4 × 4 mm round shape) with three different pore sizes (400 µm, 700 µm, and 1,000 µm, respectively) were provided by Stryker Orthopedics (USA) (Figure 2). Titanium cylinders were washed with 70% ethanol and then distilled water to remove any residue of the 3D printing powder. Titanium cylinders were then sterilized by autoclaving and dried at room temperature before use.

Animal study design

This animal study was approved by the Ascension Providence Hospital Institutional Animal Care and Use Committee, and was divided into two independent studies. Study 1 was performed to verify whether both low-grade infection (Xn29 1 × 10^3 CFU per pouch) and high-grade infection, as well as biofilm formation (Xn29 1 × 10^6 CFU per pouch), could be established in the presence of titanium cylinders with different pore sizes. A total of 18 mice were used and divided into three groups (Table I). Female mice were used in this study, as they have been used extensively in medical research and are easier to handle and to keep in harmonious groups. Data collected from female mice are expected to apply to male mice, since they share a similar body defense system.

Mice were killed one week after titanium cylinder implantation. The status of pouch infection was evaluated by microbiological and histological tests. The second study aimed to evaluate the therapeutic efficacy of saline wash plus PVA-VAN/TOB-P composite paste in the mouse pouch infection model with implantation of titanium cylinders with a 400 µm pore size. A total of 48 mice were used and divided into four groups, and each group included 12 mice (six mice for low-grade and six mice for high-grade infection) (Table II). The sample size was based on power analysis for similar published studies.

Seven days after initial implant surgery and device infection, the pouches were opened and washed with saline alone or added injection of PVA-VAN/TOB-P composites. Mice were killed 14 days after saline wash and treatment. Histology was performed on the pouch tissues and bacterial cultures on the saline washouts.

Mouse pouch infection model

Mice were quarantined for one week prior to experimentation. Air pouches were created by a subcutaneous injection of 1.5 ml of sterile air on the dorsal surface of the mice and six days allowed for pouch maturation. Under anaesthesia, intraperitoneal injection of a mixture of xylazine (10 mg/kg) and ketamine (120 mg/kg), sterilized titanium cylinders were implanted into the mature pouches through a 5 mm incision. All surgeries were performed on the same day in the following order: mice receiving the 400 µm cylinders followed by the ones receiving the 700 µm cylinders, and the last were mice receiving the 1,000 µm cylinders. Before the closure of the dorsal incision, 0.5 ml of a *S. aureus* (Xen29) suspension (1 × 10^3 CFU or 1 × 10^6 CFU) was injected into the pouch. Mice without Xen29 bacteria inoculation were included as negative controls. The incision was closed using skin glue alone or in combination with suturing using 5-0 Prolene suture materials (Ethicon, USA).

For Study 1, mice were killed one week after titanium cylinder implantation. For Study 2, surgeries were performed

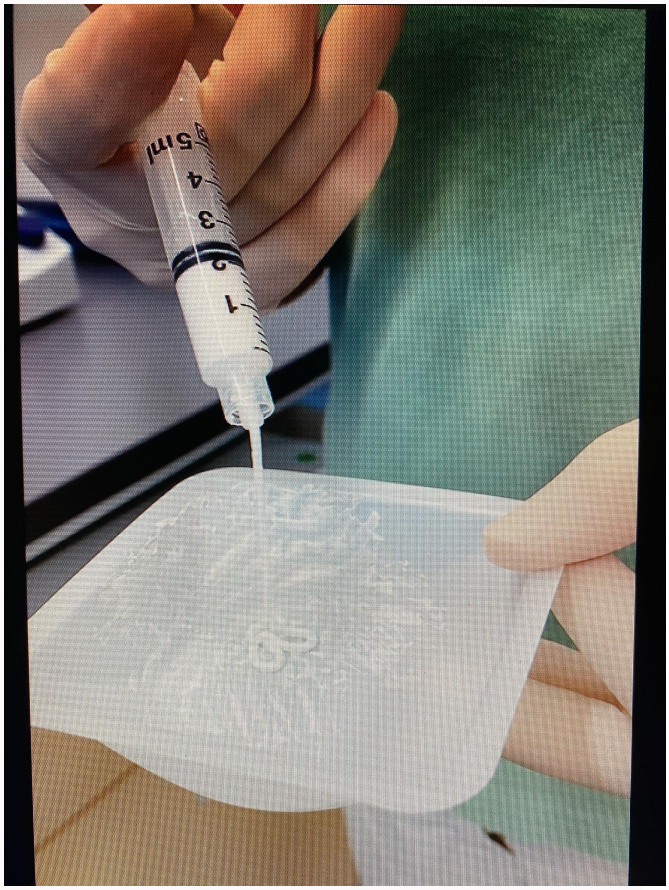

Fig. 1
A photo of the PVA-VAN/TOB-PDCPD paste showing its white colour and its injectability.

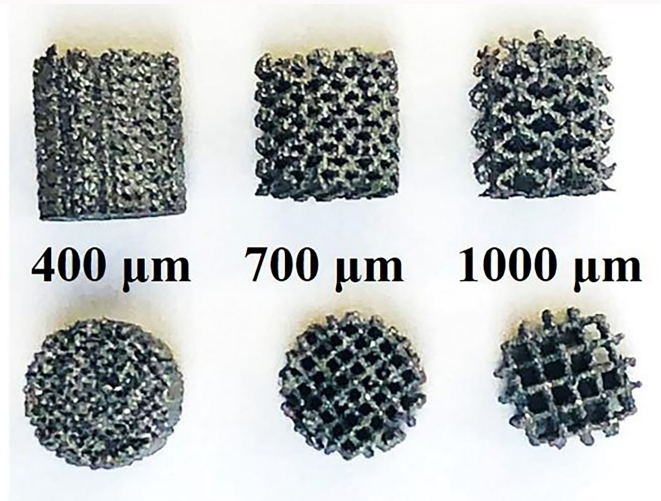

Fig. 2
Morphology and surface structure of 3D-printed titanium cylinders with well defined pore sizes.

following the group numbers, one group per day (I, II, III, and IV) with low- and high-grade infection. The pouches were opened by incision seven days after implantation, and the pouch cavity was washed with 3 ml of sterile saline. This step was done by pipetting 0.5 ml at a time into the pouch cavity followed by pipetting out the same amount; this was repeated until all 3 ml were used and then the washout was used for bacterial culture. The test materials (0.5 ml paste per pouch) were injected into the pouches after washout and before the pouch tissue and skin were closed (Group IV). Mice without saline wash (Group II) and mice with saline wash only (Group III) were included for comparison. Mice were killed 14 days after the saline washout and treatment using carbon dioxide narcosis (inhalation) and cervical dislocation. At this point, the pouch cavities were washed again with saline and the washouts collected for bacterial culture analysis. The pouch tissues and collected titanium cylinders were used for microbiological and histological analysis.

Observation and postoperative care

After surgery, mice were placed in clean cages warmed by a water circulating heating blanket and observed continuously until the effects from the anaesthetic had worn off, and were then returned to normal housing (individual). Mice were monitored daily for signs of body deterioration, pain, wound healing status, lack of eating and/or activity, and weight loss. Pain was managed by meloxicam 5 mg/kg subcutaneously if needed, and anorexia was managed with supplemental food or treats. In cases of wound dehiscence and exposure of the implant, mice were euthanized and removed from the study.

Bacterial culture of washout samples from pouch cavity and collected titanium cylinders

The washout samples collected from both pouch cavity and titanium cylinders were normalized to a total volume of 2 ml with sterile saline and stored at 4°C before analysis. A measure of 0.5 ml of the washout samples collected at given times was placed into Luria-Bertani medium containing 50 µg/ml kanamycin and cultured in a shaking incubator at 37°C for 24 hours to allow bacterial growth. The optical density (OD) at 600 nm was then measured using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, USA).

Agar plate assay of collected titanium cylinders

The explanted titanium cylinders were washed with sterile saline followed by sonication (30 seconds ×3) before agar plate testing. Luria-Bertani (LB, Thermo Fisher Scientific) agar plates containing 50 µg/ml kanamycin were prepared for quantitative and qualitative bacterial activity assay.[27] Liquid samples of the sonicates from each cylinder were plated on LB + kan plates and incubated at 37°C for 24 hours. The bacterial burden (number of CFUs formed) was quantified and recorded. After sonication, the titanium cylinders were placed on the surface of LB agar plate and incubated at 37°C for 24 hours. Qualitative analysis was recorded as bacteria still being present or not post-sonication.

SEM analysis of the titanium cylinders

Titanium cylinders removed from mice pouch cavities were fixed in 4% paraformaldehyde for 30 minutes and then dehydrated in 50%, 60%, 70%, 80%, 90%, and 100% ethanol for ten minutes each. The morphology of the titanium cylinder surface was characterized by Quanta FEG450 environmental SEM (FEI, USA). Morphologies were viewed at a 25 kV accelerating voltage.

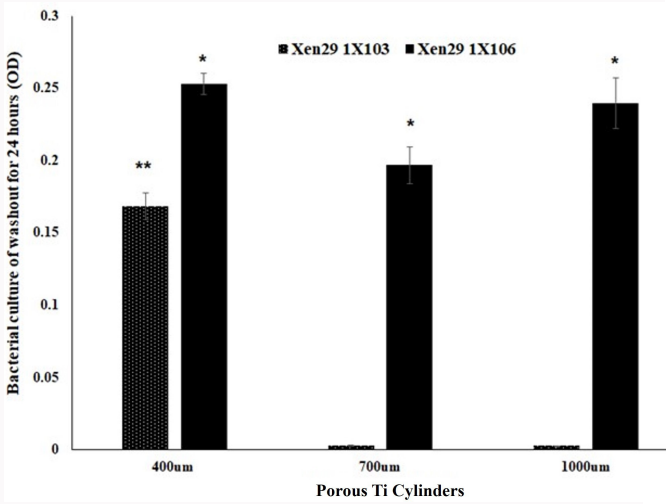

Fig. 3

Bacterial culture of pouch cavity saline washout samples seven days after titanium (Ti) cylinder implantation and Xen29 bacterial inoculation. *p < 0.05 between low-grade and high-grade inoculation of Xen29 inoculation; **p < 0.05 among Ti cylinders with different pore sizes during low-grade infection (n = 3 for each group). OD, optical density.

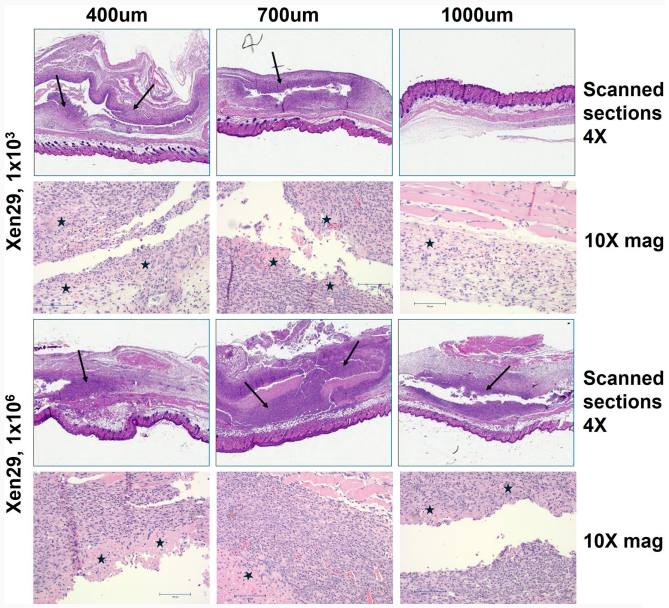

Fig. 5

Representative histological haematoxylin and eosin (H&E)-stained images of pouch tissues dissected from mice one week after implantation of contaminated porous titanium cylinders. The top two panels show lower (scanned sections) and higher magnification of the low-grade infection samples (103 CFU). The lower two panels show lower (scanned sections) and higher magnification of the high infection samples (106 CFU) for all pore sizes. Scale bars for all 10× magnification are 100 µm. Arrows indicate areas of infection and inflammation, and stars show areas of necrosis (from Study 1).

Histological assessment of pouch tissues

Pouch tissues were fixed in 10% formalin, paraffin-embedded, and stained with haematoxylin and eosin (H&E). The stained images of the whole sections were scanned with the PathScan Enabler (Meyer Instruments, USA).

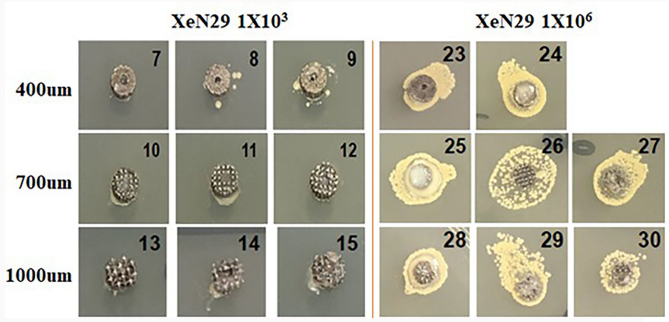

Fig. 4

Agar plate result of implanted titanium cylinders in the presence of low-grade and high-grade infection (results from Study 1).

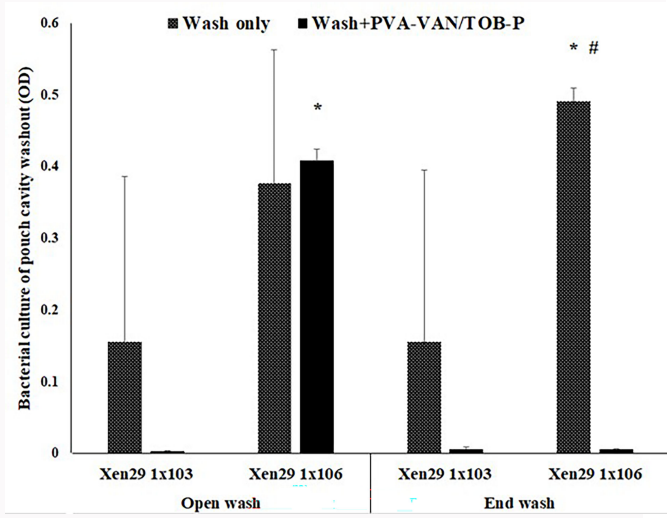

Fig. 6

Bacterial culture of pouch cavity washout during open wash and end wash between groups of saline wash only (Group III) and saline wash + polyvinyl alcohol composite (PVA)-vancomycin (VAN)/tobramycin (TOB)-dicalcium phosphate dihydrate P-DCPD (Group IV); 5% VAN-PVA gel with PDCPD particles doped with both 10% VAN and 10% TOB (n = 5 for each group). *p < 0.05 between wash only and saline wash low-grade and high-grade infection; #p < 0.05 between wash only and wash + PVA-VAN/TOB-PDCPD. OD, optical density.

Statistical analysis

OD values were expressed as mean and SD. All statistical analyses were performed using Microsoft Excel 2016 (Microsoft, USA). Data were analyzed by one-way analysis of variance with post hoc Tukey-Kramer test. Statistical significance was set to be $p < 0.05$.

Results

General observation

All animals tolerated the surgery protocol well. No mice were excluded from this study due to surgical or animal care complications. No weight loss or other signs of body deterioration were observed in any of the study groups.

Study 1

The bacterial culture results of pouch washout samples are shown in Figure 3. The low-grade infection (1×10^3 CFU) was clearly present in 400 µm titanium cylinders and, to a

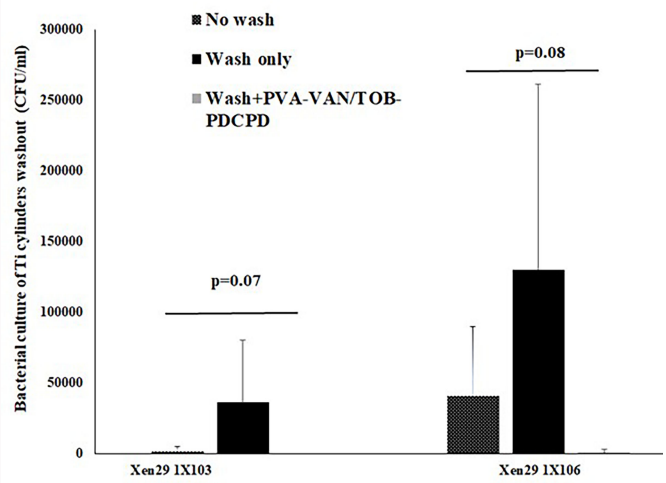

Fig. 7
Bacterial culture of titanium (Ti) cylinders washout during end wash. Group II, positive control without open saline wash; Group III, with saline wash only; Group IV, saline wash + PVA-VAN/TOB-PDCPD: 5% VAN-PVA gel with PDCPD particles doped with both 10% VAN and 10% TOB (n = 5 for each group). The p-value stands for the difference among groups using one-way analysis of variance. CFU, colony-forming units.

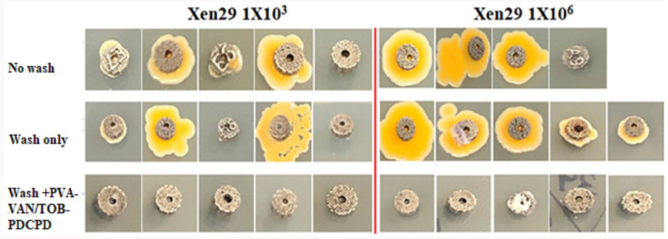

Fig. 8
Agar plate culture of Xen29 bacteria growth on harvested titanium cylinders with low- and high-grade infection among groups of positive control without open saline wash, with saline wash only, and saline wash + PVA-VAN/TOB-PDCPD (5% VAN-PVA gel with PDCPD particles doped with both 10% VAN and 10% TOB) (n = 5 for each group).

much lesser extent, in titanium cylinders with larger pore sizes (700 µm and 1,000 µm; $p < 0.001$ for both 400 µm vs 700 µm and 400 µm vs 1,000 µm). The high-grade infection (1×10^6 CFU) was observed in titanium cylinders of all three pore sizes. There was a proportional increase of bacterial growth with the increase of the bacterial inoculation in all types of titanium cylinders ($p = 0.017$ for 400 µm low vs 400 µm high, and $p < 0.001$ for both 700 µm low vs 700 µm high and 1,000 µm low vs 1,000 µm high).

Both low-grade and high-grade infections within titanium cylinders were further documented by agar plate testing (Figure 4). The bacterial growth dramatically increased at high-grade infection status compared to low-grade infection status, as seen by the increased area and density of bacterial growth around the titanium cylinders.

Both low-grade and high-grade infection and biofilm formation were established in the pouch tissue as confirmed by histological analysis of H&E-stained pouch tissues (Figure 5). However, with low-grade infections, a substantial infection was observed only in the pouches with 400 µm cylinders as manifested by severe tissue inflammation, and necrotic pouch membrane zones associated with extensive inflammatory exudation. The severity of tissue damage and inflammation were much less noticeable in the pouches with titanium cylinders with larger pore sizes (700 µm and 1,000 µm), especially in those with 1,000 µm cylinders. In high-grade infection status, substantial pouch infection and tissue inflammation were noticed in all types of titanium cylinders, but the infection was more severe in the pouches with 400 µm and 700 µm cylinders than the pouches with 1,000 µm cylinders.

Study 2

The results of bacterial culture of pouch washouts are shown in Figure 6. For the high-grade infection model, the bacterial burden for the open wash (seven days after titanium cylinder implantation and bacterial inoculation) was similar between the saline wash only group (0.38 CFU/ml (SD 0.19)) and the saline wash with PVA-VAN/TOB-P group (0.40 CFU/ml (SD 0.02)), indicating that the mouse pouch high-grade infection model was well established and reliable for the evaluation of the therapeutic efficacy of the testing materials. For the end wash (washout samples collected 14 days after open pouch saline wash), an increased bacterial burden (0.49 CFU/ml (SD 0.02)) was observed in the saline wash group compared to its previous open pouch wash result (0.38 CFU/ml (SD 0.19)), showing the progress of pouch infection. The addition of PVA-VAN/TOB-P (0.005 CFU/ml (SD 0.001)) completely eradicated bacterial retention observed in saline wash only groups ($p < 0.001$). For the low-grade infection model, a much lower bacterial burden was observed in wash with PVA-VAN/TOB-P group than that of the wash-only group for both open wash and end wash timepoints.

The bacterial culture result for washout samples from collected titanium cylinders after sonication is shown in Figure 7. Both low-grade and high-grade infection was seen within the titanium cylinders. The CFUs within Ti cylinders increased proportionally with the increase of inoculated Xen29 from 1×10^3 to 1×10^6 CFUs in both the no wash group and saline wash only group. Compared to no saline wash, the saline wash group did not decrease but slightly increased the bacterial burden, in both low-grade and high-grade infection status. When compared to the saline wash only group, the addition of PVA-VAN/TOB-P decreased bacterial growth both in low-grade ($p = 0.077$) and high-grade infection ($p = 0.084$) models.

The therapeutic efficacy of PVA-VAN/TOB-P was further supported by agar plate culture assay. As shown in Figure 8, bacterial growth around titanium cylinders was completely cleaned in wash plus PVA-VAN/TOB-P groups.

In addition, SEM analysis demonstrated the bacterial growth and biofilm formation within the titanium cylinders in both the low- and high-grade infections. The addition of PVA-TAN/TOB-P led to a remarkable decrease in the bacteria seen on the surface of the porous titanium cylinders, especially for the low-grade infection model (Figure 9).

Histological analysis of paraffin-embedded pouch tissue specimen is shown in Figure 10. At low-grade infection status, as compared to negative control, severe hyperaemia and purulence were induced in pouches without wash (Group II), which was somewhat worsened by saline wash (Group III). The degree of hyperaemia and purulence observed in Groups

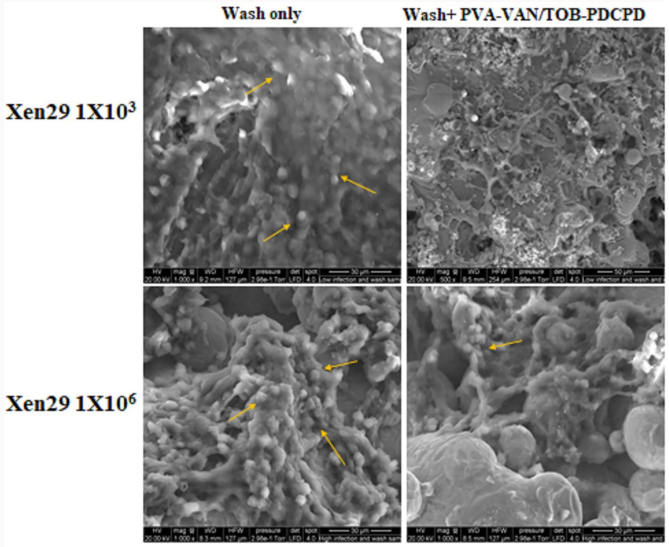

Fig. 9
Comparison of scanning electron microscopy morphology of titanium cylinder surface between wash only and wash with PVA-VAN/TOB-PDCPD (n = 1 from each group). Arrows indicate the accumulation of bacterial cluster and biofilm formation.

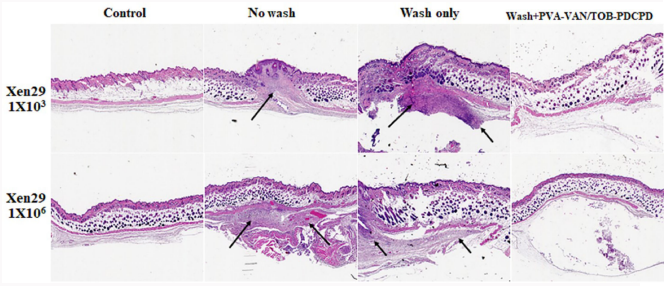

Fig. 10
Representative histological images of haematoxylin and eosin-stained pouch tissues dissected from mice two weeks after open pouch wash with and without vancomycin/tobramycin-doped polyvinyl alcohol composite (PVA-VAN/TOB)-polymeric dicalcium phosphate dehydrate composite (PDCPD) gel paste implantation (magnification at ×4). Group I, control; Group II, positive control without saline wash; Group III, with saline wash only; Group IV, saline wash + PVA-VAN/TOB-PDCPD: 5% VAN-PVA gel with PDCPD particles doped with both 10% VAN and 10% TOB. Arrows indicate the tissue inflammation and infection.

II and III was greatly improved by the additional application of PVA-VAN-TOB-P (Group IV). For the high-grade infection model, a considerable level of infection was observed in the no wash and wash only groups; this was manifested by severe tissue inflammation and necrotic pouch membrane zones associated with extensive inflammatory exudation. The tissue inflammation and infection status were completely cleaned by PVA-VAN/TOB-P treatment.

The histological findings were in good agreement with the results of the bacterial cultures of washouts (Figures 6 and 7), agar plate test (Figure 8), and SEM findings (Figure 9). In addition, no ceramic residues of testing materials were found in the pouch tissues or the pouch washout samples of Group IV, indicating that the implanted materials were completely degraded 14 days after injection.

Discussion

Implant-associated infection remains a leading cause of implant failure and revision.[28,29] Saline irrigation for treatment of acute or chronic PJI has limited efficacy,[30] likely because of retention of bacteria and biofilm adherent to the implanted medical devices.[31] However, DAIR is clinically desirable and frequently employed. Therefore, more effective and user-friendly approaches focused on long-term wound protection and eradication of remnant/retained bacteria are clinically desired.

Data from Study 1 demonstrated that both low-grade (1×10^3 CFU) and high-grade (1×10^6 CFU) *S. aureus* inoculation induced pouch infection and biofilm formation in the presence of porous titanium cylinders without adverse effects to the mouse body. Two different loading amounts of *S. aureus* (1×10^3 CFU vs 1×10^6 CFU per pouch) were chosen based on previous studies, and to mimic the clinical scenarios of low-grade and high-grade infection with biofilm formation.

It was reported that the severity and persistence of implant-associated infection are influenced by the size, surface roughness, and porosity of implanted medical devices.[2,19] As shown in Figure 3, the low-grade infection was more obvious in the 400 µm titanium cylinders than in titanium cylinders with larger pore sizes (700 µm and 1,000 µm, $p < 0.001$). The difference in infection severity between the titanium cylinders was further supported by histological analysis (Figure 5). A substantial infection was observed only in pouches with 400 µm cylinders as manifested by severe tissue inflammation, and necrotic pouch membrane zones associated with extensive inflammatory exudation. The severity of tissue damage and inflammation were much less noticeable in the titanium cylinders with larger pore sizes (700 µm and 1,000 µm), especially for those with 1,000 µm pore sizes. We propose that this finding may be, at least in part, due to the reduced bacterial adherence caused by the reduction of matrix surface area with the increased pore size. Data from Study 1 validated the two clinically relevant low- and high-grade pouch infection models. Since a 400 µm pore size would be commonly found among implants in a clinical setting, and was more susceptible to both the low- and high-grade infections, we chose the 400 µm titanium cylinders for use in Study 2.

In Study 2, we compared the therapeutic efficacy of saline wash alone versus saline wash plus injection of a PVA-VAN/TOB-P composite paste. Some studies demonstrated that saline wash observably reduced the bacterial burden and improved, in part, the infection status in the presence of contaminated sutures.[2,3] Data from the current study, however, did not show efficacy of the saline washes. The bacterial burden and severity of tissue infection appeared to worsen after the saline wash in both the low-grade and high-grade infection models compared to the no saline wash control (Figure 5, 6, and 10). This may be due, at least in part, to the increased size, volume, and porous matrix of titanium cylinders compared to sutures. It might also be caused by the weak mechanical force and limited time used for saline wash within the pouch cavities.

Our data demonstrated that PVA-VAN/TOB-P was effective in eradicating porous implant-associated low- and high-grade infections, as shown by both the negative results

of bacterial culture of pouch washouts (Figure 6) and effective infection control and disappearance of histological tissue inflammation and purulence (Figure 10). The therapeutic efficacy of PVA-VAN/TOB-P was further supported by the agar plate culture assays (Figure 8). Lastly, SEM analysis demonstrated bacterial growth and biofilm formation within titanium cylinders only in the saline wash group. The addition of PVA-TAN/TOB-P effectively cleaned the infection even in the presence of a porous metallic titanium implant, especially in the low-grade infection model (Figure 9). Concordant with the microbiological findings, histological analysis showed that the pouch tissue inflammation and infection were completely eradicated by the PVA-VAN/TOB-P treatment (Figure 9). The observed in vivo efficacy of PVA-VAN/TOB-P was due to its distinctive release pattern of vancomycin and tobramycin.[22] The biphasic model of early burst vancomycin release with a sustained release of tobramycin afterwards found in VAN/TOB-P is of clinical benefit. An early burst release of vancomycin at higher concentration immediately after material implantation provides sufficient inhibition of bacterial growth at surgical infection sites, while the slow and sustained tobramycin release thereafter prevents residual bacterial growth and biofilm formation, and minimizes any local or systemic toxicity associated with high fluctuating antibiotic concentrations.

PVA-VAN/TOB-P was biocompatible and did not initiate tissue inflammation and/or foreign body reaction (Figure 10). PVA is a Food and Drug Administration (FDA)-approved polymer that is biocompatible.[32] The P-DCPD fine particles were non-toxic and have not shown inhibition of osteoblastic cell growth even at higher concentrations (100 mg/ml).[33] Vancomycin and tobramycin were the least cytotoxic antibiotics studied based on in vitro osteoblastic cell viability studies.[34] Additionally, VAN/TOB-P were non-toxic and had no negative impacts on the growth of osteoblastic MC3T3 cells.[22] Data from the present study further confirmed the in vivo safety of PVA-VAN/TOB-P composites when all these components were mixed. In addition, PVA-VAN/TOB-P was biodegradable.[22,35] Blending of PVA gel with P-DCPD ceramic particles had little impact on the degradation of these components. In summary, data from Study 2 confirmed that the saline irrigation was not effective in the presence of porous titanium cylinders, whereas the addition of a biodegradable and biocompatible PVA-VAN/TOB-P was very effective in eradicating bacteria.

Future clinical application of PVA-VAN/TOB-P composite as a paste or a powder is promising. The results from this study confirm the merit of PVA-VAN/TOB for the treatment of implant-related infection after saline irrigation. Our next step is to test the efficacy of PVA-VAN/TOB in the prevention of such infection by implanting titanium cylinders pre-coated with PVA-VAN/TOB into a contaminated mouse pouch. A more advanced step will be mimicking the DAIR procedure in an animal model by implanting the titanium cylinders into the knee joint, infecting it, and then treating it with the studied material.

There were some limitations to this study. First, SEM analysis of bacterial adherence and biofilm formation was descriptive rather than quantitative. Second, no quantitative measurements of biofilm mass in the washout samples or within titanium cylinders were performed. Third, only one bacterial strain (*S. aureus*) was used for testing. More studies would be needed to establish efficacy against other relevant pathogens in the face of a porous implant-associated infection. Other limitations of the study are the use of only female mice and a lack of animal randomization. Blinding to treatment was difficult since the surgeries were performed by a single surgeon (BW) and the tested material is physically different from the control. Additionally, the results from this work cannot be directly transferred to periprosthetic joint infection.

Porous implant-associated infections are common and yet difficult to treat. We validated a mouse model for low-grade and high-grade pouch infection with implanted 3D porous titanium cylinders without deterioration of the mice's general health. Our data showed that saline irrigation efficacy was poor in the presence of contaminated titanium cylinders. PVA-VAN/TOB-P was biodegradable, biocompatible, and highly effective at eradicating bacteria after saline wash despite the presence of contaminated titanium cylinders in both low-grade and high-grade infection models. Application of PVA-VAN/TOB-P directly into the surgical site alone or after saline wash represents a feasible and cost-effective option for treatment during a DAIR procedure. Further material optimization and validation in large animals are required to achieve a clinically applicable product.

Supplementary material

ARRIVE checklist.

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Data sharing

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Ethical review statement

This animal study was approved by the Institutional Animal Care and Use Committee of Ascension Providence Hospital (Southfield, Michigan, USA) (IACUC 104-17).

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