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Antimicrobial properties of antibiotic-loaded implants

A COMPARATIVE STUDY OF ANTIMICROBIAL PROPERTIES OF ANTIBIOTIC-LOADED CEMENT, TANTALUM, 3D-PRINTED POROUS TITANIUM, AND TITANIUM ALLOY

Aims

The aim of this study was to compare the ability of tantalum, 3D porous titanium, antibiotic-loaded bone cement, and smooth titanium alloy to inhibit staphylococci in an *in vitro* environment, based on the evaluation of the zone of inhibition (ZOI). The hypothesis was that there would be no significant difference in the inhibition of methicillin-sensitive or methicillin-resistant *Staphylococcus aureus* (MSSA/MRSA) between the two groups.

Methods

A total of 30 beads made of three different materials (tantalum/3D porous titanium and smooth titanium alloy) were bathed for one hour in a solution of 1 g vancomycin in 20 ml of sterile water for injection (bath concentration: 50 mg/mL). Ten 1 cm³ cylinders of antibiotic-loaded cement were also created by mixing standard surgical cement with 1 g of vancomycin in standardized sterile moulds. The cylinders were then placed on agar plates inoculated with MSSA and MRSA. The ZOIs were measured each day and the cylinders were transferred onto a new inoculated plate.

Results

For MSSA and MRSA, no inhibitory effect was found in the control group, and antibiotic-loaded smooth titanium alloy beads showed a short inhibitory effect until day 2. For MSSA, both tantalum and 3D porous titanium beads showed significantly larger mean ZOIs than cement beads (all $p < 0.01$) each day until day 7 for tantalum and until day 3 for 3D porous titanium. After six days, antibiotic-loaded cement had significantly larger mean ZOIs than the 3D porous titanium ($p = 0.027$), but no significant difference was found with tantalum ($p = 0.082$). For MRSA, both tantalum and 3D porous titanium beads had significantly larger mean ZOIs than antibiotic-loaded cement each day until day 6 for tantalum (all $p < 0.01$) and until day 3 for 3D porous titanium (all $p < 0.04$). Antibiotic-loaded cement had significantly larger mean ZOIs than tantalum and 3D porous titanium from day 7 to 9 (all $p < 0.042$).

Conclusion

These results show that porous metal implants can deliver local antibiotics over slightly varying time frames based on *in vitro* analysis.

Introduction

Prosthetic joint infection (PJI) remains the main cause of failure in primary and revision total knee arthroplasty (TKA).^{1,2} Local delivery of antibiotics, mainly using antibiotic-loaded bone cement,³ is commonly used in the management of PJI. Over the past decade, tantalum^{4,5} and porous titanium^{6,7} have been successfully used as metaphyseal devices to

address bone loss and improve biological fixation at revision TKA. As their use involves a decrease in the amount of antibiotic-loaded cement required at the surgical site, previous authors have investigated the antimicrobial properties of intrinsic^{8,9} and/or artificially created antibiotic-loaded porous materials.^{10,11} In two previous studies we have shown that tantalum does not have any intrinsic antimicrobial

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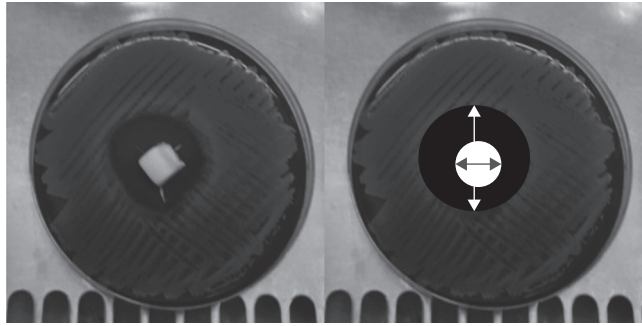


Fig. 1

Measurement of the zone of inhibition. The calculation is based on the following equation: $ZOI = \text{Raw ZOI (blue circle)} - \text{specimen's diameter (green circle)}$.

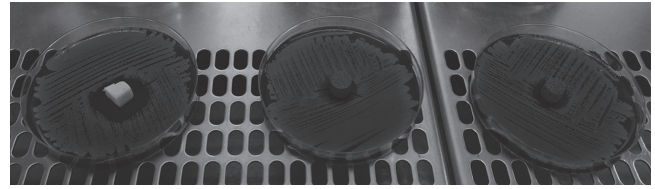
properties but is able to deliver vancomycin in both liquid and solid environments.^{10,11}

To date, no study has examined the antimicrobial properties of antibiotic-loaded 3D-printed porous titanium (3DT) and compared it with bone cement. The aim of this study was to compare the ability of tantalum, 3DT, antibiotic-loaded cement, and smooth titanium alloy to inhibit staphylococci in an in vitro environment, based on the evaluation of the zone of inhibition (ZOI) and the duration of antibacterial activity. The hypothesis was that there would be no significant difference in the inhibition of methicillin-sensitive (MSSA) or methicillin-resistant *Staphylococcus aureus* (MRSA) between the two groups.

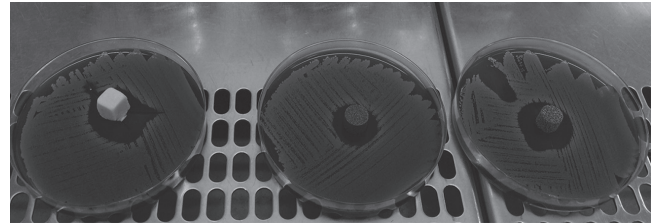
Methods

In order to study the antibacterial properties of various alloys, ten 3D-printed porous titanium (Tritanium; Stryker, Mahwah, New Jersey, USA) cylinders (1 cm³, 4 g mean pore size 400 to 500 μm porosity of 55% to 65%), ten trabecular metal (Tantalum; Zimmer-Biomet, Warsaw, Indiana, USA) cylinders (1 cm³, 4 g mean pore size 400 μm to 500 μm, porosity 75% to 80%) and ten smooth titanium alloy (Ti-6Al-4V ELI Alloy; Medtronic, Dublin, Ireland) cylinders (1 cm³, 4 g) were supplied by the manufacturers. As we aimed to reproduce 'clinical implants', all cylinders were created using the same protocol as those used to design augments, sleeves, or cones used at arthroplasty. All cylinders were sterilized by the manufacturers and shipped in individual packages.

Ten antibiotic-loaded cement cylinders measuring 1 cm³ (Palacos; Heraeus Kulzer GmbH, Wehrheim, Germany) were constructed as a control group. The preparation of the cylinders was standardized using identical sterile moulds, filled with sterilized and prepackaged cement. In particular, one 40 g batch of polymethylmethacrylate (PMMA) was used to which 1 g of vancomycin powder was added. Cement and vancomycin were mixed using a dedicated vacuum (Palamix; Heraeus Kulzer GmbH, Wehrheim, Germany) and following the recommendations of the supplier; 20 ml of liquid monomer was then added and mixed for 60 seconds. While still doughy, loaded cement preparations were used to fill dedicated moulds and create ten 1 cm³ beads.



a



b

Fig. 2

a) Zone of inhibition (ZOI) analysis (Day 7) regarding vancomycin-loaded specimens and methicillin-resistant *Staphylococcus aureus*.
b) ZOI analysis (Day 7) regarding vancomycin-loaded specimens and methicillin-sensitive *S. aureus*. 3DT, 3D-printed porous titanium; ALBC, Antibiotic-loaded bone cement.

Soaking solutions were prepared by dissolving and diluting the antibiotics as follows: 1 g of vancomycin (Sandoz; Holzkirchen, Germany) in 20 ml of sterile water (pH 5.5, osmolarity 0 mOsmol/l) for injection (CDM Lavoisier, Paris, France), resulting in a concentration of 50 mg/ml. Then, each cylinder was soaked in an individual beaker with antibiotic solution for one hour at room temperature. Samples were tested for MSSA and MRSA inhibition using a medium diffusion bioassay as previously described.^{10,11} Each specimen (metal and cement cylinders) was placed using sterile forceps on an agar plate inoculated with either MSSA or MRSA, and then incubated at 37 °C for 24 hours. Each cylinder was withdrawn daily and put on a new agar plate to simulate a new bacterial agents' attack. This step was repeated each day until no antibiotic activity was found. All manipulations were performed under a hood.

Each day, the antibiotic ZOI was recorded with diameters measured in millimetres using a digital vernier caliper and with an automated computed evaluation (Scan 1200-Interscience, Saint-Nom-la-Bretèche, France), to assess intra- and inter-observer reproducibility. The diameter of the porous tantalum and cement cylinders was subtracted from the total ZOI (Figure 1). In order to assess reproducibility the measurements of the ZOIs were repeated twice by the same observer (intraobserver) and compared with automatic measurement (interobserver). High-definition photographs of each sample were made every day. The intraclass correlation coefficients (ICCs) indicated a nearly perfect intra- and interobserver reproducibility for the measurement of ZOI (ICC 0.96 IC 95% [0.93 to 1] and ICC 0.98 IC 95% [0.96 to 1], respectively).

Statistical analysis. Parameters of interest were expressed as mean and SD. Differences between groups were evaluated using a non-parametric (Wilcoxon signed-rank) test due to our

Table I. Mean ZOI (mm) measured every day on a new agar plate inoculated with methicillin-sensitive *Staphylococcus aureus*.

| Material | J1 | J2 | J3 | J4 | J5 | J6 | J7 | J8 | J9 | J10 | J11 | J12 |
|---------------|--------------|--------------|--------------|--------------|--------------|--------------|-------------|-------------|-----------|-------------|-------------|-------------|
| Tantalum (SD) | 32.6 (1.47) | 23.8 (0.69) | 22.62 (2.95) | 17.02 (5.31) | 16.50 (3.14) | 11.88 (7.58) | 9.56 (7.21) | 6.14 (6.63) | 0 | 0 | 0 | 0 |
| 3DT (SD) | 32.94 (5.29) | 22.7 (2.08) | 17.04 (2.21) | 11.7 (1.92) | 10.9 (2.15) | 1.96 (4.38) | 1.18 (2.63) | 0 | 0 | 0 | 0 | 0 |
| STA (SD) | 30.3 (4.52) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ALBC (SD) | 20.33 (0.8) | 14.92 (2.38) | 14.04 (4.92) | 10.98 (1.12) | 10.4 (3.24) | 14.46 (5.72) | 8.66 (6.12) | 4.64 (4.24) | 4.2 (6.6) | 1.02 (2.28) | 1.74 (3.89) | 1.20 (2.68) |

*DT, 3D-printed porous titanium; ALBC, Antibiotic-loaded bone cement; STA, smooth titanium alloy.

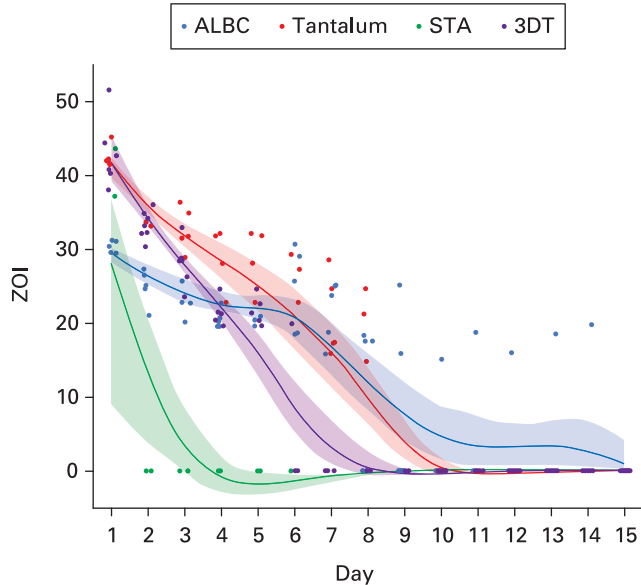


Fig. 3

Zone of inhibition analysis regarding methicillin-sensitive *Staphylococcus aureus* adhesion. 3DT, 3D-printed porous titanium; ALBC, Antibiotic-loaded bone cement; STA, smooth titanium alloy.

sample size. Based on previously reported ZOIs of antibiotic-loaded cement, our sample size was sufficient to distinguish differences > 3 mm with an expected standard deviation of 2 mm and a statistical power $> 90\%$.¹²

Results

For MSSA and MRSA, no inhibitory effect was found in antibiotic-loaded smooth titanium alloy, having a short inhibitory effect until day 2 only.

For MSSA, both Tantalum and 3DT beads had statistically significantly larger mean ZOIs than cement beads (all $p < 0.01$) each day until day 7 for tantalum and until day 3 for 3DT (Figure 2). After six days, antibiotic-loaded cement had a larger mean ZOI than 3DT ($p = 0.027$), but no significant difference was found with tantalum ($p = 0.082$) until no inhibitory effect occurred at day 12 (Table I and Figure 3).

For MRSA, both tantalum and 3DT beads had statistically significantly larger mean ZOIs than antibiotic-loaded cement each day until day 6 for tantalum (all $p < 0.01$) and until day 3 for 3DT (all $p < 0.04$) (Figure 2). Antibiotic-loaded cement had larger mean ZOIs than tantalum and 3DT between day 7 and 9

(all $p < 0.04$) but not after nine days, until no inhibitory effect occurred at day 12 (Table II and Figure 4).

Discussion

The hypothesis of our study was confirmed. First, porous implants can deliver antibiotics to a surgical site; secondly, this ability provided tantalum implants with an antibacterial potential equivalent to that of antibiotic-loaded cement in the treatment of MSSA; thirdly, for both tantalum and 3DT this antibacterial potential was substantial but less than that of antibiotic-loaded cement in the treatment of MRSA; and finally, tantalum-loaded cylinders inhibited staphylococcal adhesion for a longer period than 3D-printed and antibiotic-loaded porous titanium implants. Since porous metal implants are increasingly being used at revision THA and TKA, local antibiotics delivered using the simple soaking process described in this study might play a role in mitigating the risk of PJI. However, an advantage of local antibiotics is that they can be administered at much higher concentrations than is achievable parenterally, without the associated systemic toxicity.^{3,13-15} Antibiotics may also be delivered in this way into avascular areas which are inaccessible using parenteral antibiotics.^{16,17} These data allow us to believe that future antibiotic-loaded porous constructs will help surgeons decrease the rates of infection and reinfection in revision TKA.¹⁸

This study has limitations. The in vitro model of the in vivo release of antibiotics from porous tantalum and antibiotic-loaded cement only approximated to the behavior of vancomycin released into synovial fluid. The diffusion medium does not fully represent the complex in vivo environment and we only evaluated the antimicrobial properties of vancomycin-soaked porous implants on nearby staphylococci. Thus, we cannot draw conclusions about a more distant effect or about local antibiotic pharmacokinetics. Secondly, we were unable to determine the exact concentration of vancomycin on the cylinders before they were placed on the agar plates. We assumed that each cylinder was soaked in an identical solution containing the same amount of vancomycin (50 mg/ml). Thirdly, the process described in this study is an off-label use of both porous implants and vancomycin powder. We did not investigate the potential deleterious effects of the integration of vancomycin implants on bone ingrowth. Further study focusing on the ideal timing of the soaking period is needed to evaluate the concentration/dose effect of a more clinically relevant length of time (five to 15 minutes).

We, however, think that this method is robust. It has been validated in one of our previous studies.¹⁰ All measurements

Table II. Mean ZOI (mm) measured every day on a new agar plate inoculated with methicillin-resistant *Staphylococcus aureus*.

| Material | J1 | J2 | J3 | J4 | J5 | J6 | J7 | J8 | J9 | J10 | J11 | J12 |
|---------------|--------------|---------------|--------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-----|
| Tantalum (SD) | 41.86 (4.76) | 24.62 (1.16) | 24.5 (1.64) | 16.8 (1.18) | 15.3 (1.887) | 6.42 (3.62) | 2.60 (3.56) | 2.33 (1.45) | 0 | 0 | 0 | 0 |
| 3DT (SD) | 38.86 (5.52) | 22.78 (6.94) | 16.88 (3.95) | 11.22 (4.45) | 9.58 (4.36) | 5.08 (3.34) | 3.52 (3.68) | 0 | 0 | 0 | 0 | 0 |
| STA (SD) | 51.25 (4.17) | 8.20 (0.42) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| ALBC (SD) | 24.42 (5.31) | 19.32 (4.883) | 11.04 (3.16) | 8.52 (4.3) | 8.28 (4.98) | 9.76 (5.81) | 8.80 (5.12) | 7.29 (5.09) | 5.56 (5.13) | 3.40 (5.27) | 6.28 (8.68) | 0 |

3DT, 3D-printed porous titanium; ALBC, Antibiotic-loaded bone cement; STA, smooth titanium alloy.

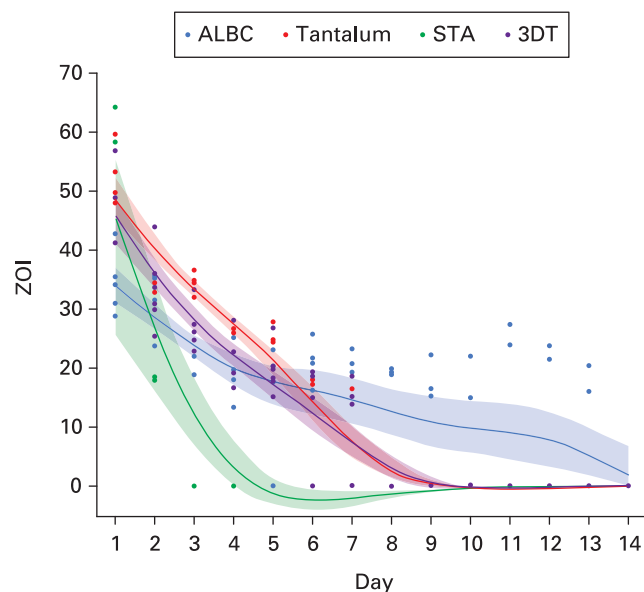


Fig. 4

Zone of inhibition analysis relating to methicillin-resistant *Staphylococcus aureus* adhesion. 3DT, 3D-printed porous titanium; ALBC, Antibiotic-loaded bone cement; STA, smooth titanium alloy.

were done using manual and automated methods to enhance the precision of our data collection. As most of the methodological part of the study follows the previously validated protocol, we did not include control cylinders. We have previously shown that Tantalum has no intrinsic properties which inhibit bacterial adhesion.¹⁰

Han et al¹⁹ were the first to investigate the microarchitectural advantages of a porous metal alloy, using chitosan-coated bovine serum albumin nanoparticles and oxidized alginate in a layer-by-layer manner fixed on porous titanium scaffolds. They showed that the nanostructures of their specimens were able to deliver growth factors and antibacterial agents (vancomycin) in a liquid growth environment. Their methods are promising even if the engineering and manufacturing of specimens may not easily be used clinically. Following this initial discovery of the properties of a porous alloy a few authors have investigated the antimicrobial properties of antibiotic-loaded implants. Chang et al²⁰ described an in vitro study of antibiotic-loaded cement prepared from 1 g of vancomycin per 40 g of PMMA for inhibition of MSSA. They reported a two-day antibacterial effect, but their protocol was based on dilution bioassay. Using the same analysis Yuenyongviwat et al¹² reported inhibitory effects of vancomycin-loaded cement on MRSA adhesion for four weeks. These results contrast with our maximal 12-day inhibition for

antibiotic-loaded cement. First, the concentrations of vancomycin in the three protocols were different (4 g vancomycin for one articulating spacer vs 1 g for all our cylinders). We thought that only a drop of antibiotic could inhibit bacteria on as small a surface as an agar plate. We preferred to use a solid loaded implant that could be used to treat staphylococci on a solid medium, day after day. We believe that this protocol permits closer estimations of in vivo conditions.

We estimated, with a method similar to this study protocol, antimicrobial properties of tantalum cylinders and antibiotic-loaded cement¹⁰. We found that porous tantalum cylinders inhibited MSSA for up to 12 days, whereas cement cylinders inhibited MSSA for nine days. Porous tantalum cylinders had statistically significantly larger mean ZOIs than the antibiotic-loaded cement each day until day 11. These results are similar to those in this further study for the first ten days. Antibiotic-loaded cement cylinders, however, inhibited MSSA adhesion for a longer period in this study. We think that this difference might be explained by the method which we used in this study to mix vancomycin with cement in a dedicated vacuum. Recently, Gergely et al²¹ highlighted the advantages in terms of antibiotic and cement distribution in the final product using vacuumed cement preparation. As such, the equal distribution of antibiotics in all cylinders might have positively affected the duration of antimicrobial inhibition described in this study.

Finally, if porous implants exhibited antibacterial properties similar to those of antibiotic-loaded cement when treating MSSA, vancomycin-loaded cement allowed longer antimicrobial potential than tantalum and 3DT when treating MRSA. This three-day difference might be clinically detrimental and an additional study should be conducted using a higher soaking concentration and/or different antibiotics.

In conclusion, we found that porous metal implants can deliver local antibiotics over slightly varying time frames based on in vitro analysis. Antibiotic-loaded tantalum and 3DT constructs showed superior antimicrobial properties when compared with smooth titanium alloy. Future goals include impregnating porous metals with antibiotics for intraoperative use during revision TKA.

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