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Characterization of Ultrasound-Triggered Bulk Antibiotic Release from Novel Spinal Hardware

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Abstract*— This study evaluated the efficacy of ultrasound-triggered drug delivery devices against bacterial infection in an *ex vivo* cadaveric rabbit spine model. Polylactic acid (PLA)-coated, vancomycin (VAN)-loaded polyether ether ketone (PEEK) devices (1 cm^3) with a drug-loading reservoir (0.785 cm^3) were 3D printed. Two device designs were evaluated: 1 large hole for drug release vs 2 smaller holes. Clips were implanted medial to the spinal midline in mature (~6 months, 3 kg) female White New Zealand cadaveric rabbits (n=4) under an IACUC-approved protocol. To simulate infection, 10^4 CFU of *Staphylococcus aureus* were added to 2 of the 4 sites; the other 2 sites were left clean. Two of the 4 sites (1 inoculated, 1 clean) were insonated for 20 minutes with a Logiq E9 ultrasound scanner (GE Healthcare, Waukesha, WI, USA) equipped with a C1-6 curvilinear probe, using power Doppler imaging (1.7 MHz frequency, 6.4 kHz PRF, 100% acoustic output power) to induce rupture of the PLA coating for VAN release. Infected sites showed marked reduction in bacterial colonization following ultrasound-triggered VAN release, while uninsonated sites exhibited little reduction in bacterial colonization. At 48 hours, there was significantly greater VAN release from the insonated clips compared to the uninsonated clips ($p < 0.04$). There was significantly greater ultrasound-triggered total VAN release from the 1-hole device design than from the 2-hole design (7420 ± 2992 μg vs. 3500 ± 954 μg, $p < 0.0001$). These levels are sufficient to prevent adhesion of *S. aureus* to implant materials. This study demonstrated the feasibility of an ultrasound-mediated antibiotic delivery device, which could become a potent weapon against spinal surgical site infections.

Keywords— ultrasound triggered delivery, spine, infection

I. Introduction

Bacterial infection following spinal fusion surgery is a major clinical concern, with up to 10% of patients developing infection despite aggressive peri-operative antibiotic treatments [1]. Currently, the clinical standard of prophylactic care in spinal fusion surgery is to sterilize the surgical site with 1-2 g of powered vancomycin (VAN) during wound closure [2]. However, this method has variable outcomes due to several factors, including wound drainage, patient health, and the presence of Gram-negative bacteria. Additionally, pathogens that were not initially treated or eradicated by the powdered VAN may overgrow and develop into a post-operative infection. Research suggests that these infections persist due to bacterial colonization and biofilm formation on the spinal instrumentation (e.g., metal screws and rods, as well as cages made from polyetheretherketone (PEEK)) [3, 4]. In the spine, the instrumentation exists at the bone/muscle interface, and is bathed in post-operative wound fluid that influences bacterial formation of biofilms [5-7]. Biofilms exhibit reduced response to antibiotics, complicating treatment of post-operative infection [8]. Once such a recalcitrant infection develops, treatment often requires reopening the surgical site, and in extreme cases removal of the spinal fusion hardware followed by additional revision surgeries. With only partially effective peri-operative antibiotic treatments, development of more effective means to prevent infection is both an economic and clinical imperative.

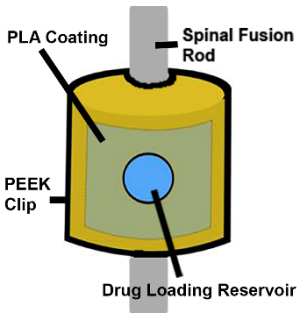

Fig. 1. Representative image of 1-hole PEEK clip for US-triggered drug delivery (not to scale).

To combat this problem, we have designed an ultrasound (US) activated drug release system to deliver prophylactic antibiotics to combat post-surgical bacterial survival (Figure 1) [9]. Our hypothesis is that maintaining supra-therapeutic concentrations of prophylactic antibiotics at the hardware site following spinal fusion surgery will lower post-operative infection rates. To evaluate this hypothesis, we have developed a polyether ether ketone (PEEK) spacer clip that is mounted onto the spinal fusion rod, and is sealed with polylactic acid (PLA) to serve as a non-eluting reservoir of combination

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antibiotics (Figure 1) [9]. The goal of this study was to quantify antibiotic release from these devices and to evaluate their efficacy against bacterial infection in an *ex vivo* rabbit model.

II. Materials and Methods

A. Device Preparation and Evaluation

PLA-coated, VAN-loaded PEEK clips (1 cm^3) with a drug-loading reservoir (0.785 cm^3) were created in our labs. Briefly, the PEEK clips were designed using Solidworks 2016 (Dassault Systemes, Vélizy-Villacoublay, France), exported as a stereolithography (STL) code, converted to a G-code (Simplify3D, Cincinnati, OH, USA), and printed using a PEEKMed filament on an Indmatec HPP 155/Gen 2 3D printer (Apium Additive Technologies, Karlsruhe, Germany). Two designs were evaluated: (1) a semi-circular clip with one 4 mm diameter opening to the reservoir for loading/release (1-hole), and (2) a semi-circular clip with two 2 mm openings (2-hole). Clips were loaded until filled with a VAN solution (50 mg VAN/device, Athenex, Buffalo, NY, USA) and then painted with a solution of PLA (Resomer Select 100 DL 7E, Evonik Industries, Essen, Germany) and dried overnight to create a thin coating to seal the reservoir.

US-triggered antibiotic release was quantified following insonation with a Logiq E9 ultrasound scanner (GE Healthcare, Waukesha, WI, USA) equipped with a C1-6 curvilinear probe, using power Doppler imaging (1.7 MHz frequency, 6.4 kHz PRF, 100% acoustic output power). Coated, antibiotic-loaded pucks were submerged in 5 ml trypticase soy broth (TSB) containing 10^5 CFU/ml *Staphylococcus aureus*. Individual clips were insonated for 10 minutes. At both 1 and 24 hours after insonation, antibiotic release was determined by sampling 50 µL from each well, followed by spotting and drying onto a BD BBL blank Sensi-Disk™ (Becton Dickinson, Franklin Lakes, NJ, USA). The disc was then placed onto a TSB agar plate containing a lawn of *S. aureus*, and, after overnight incubation at 37˚C, the zone of inhibition due to VAN diffusion measured [10]. VAN amounts were estimated by comparison to a standard curve of VAN concentrations as well as to a commercial 30 mg VAN standard disc [11, 12]. Clip-adherent bacteria were measured at 24 hours Controls included (1) coated, loaded pucks that were not insonated, (2) loaded, uncoated pucks with and without insonation, and (3) bacteria only with and without insonation. Results were collected in triplicate and experiments repeated at least three times.

For *ex vivo* testing, clips were implanted medial to the spinal midline in cadaveric, mature (~6 months, 3 kg), female White New Zealand rabbits (Covance, Princeton, NJ, USA, n = 4) under an IACUC approved protocol. 10^4 CFU *S. aureus* were added to 2 of the 4 sites, and 2 sites were left as was. Wounds were closed with Proline 2.0 suture (Ethicon, Somerville, NJ, USA). Two of the 4 sites were insonated for 20 minutes. In parallel, positive and negative bacterial controls were evaluated, where the surgical procedure was performed without implantation of PEEK clips. All implanted devices and control sites were incubated for 2 hours post-insonation, then retrieved for analysis. Results were collected in duplicate for each evaluated device and condition.

B. Statistical Analysis

Statistical analysis was performed with GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA), with p-values below 0.05 indicating statistical significance. Comparisons between clip designs and groups were performed using a one-way ANOVA with Bonferroni correction for multiple comparisons and Tukey's multiple comparison post-test when appropriate. Differences were evaluated across all groups, and also within each group for more robust analysis. Error bars represent standard deviation (SD).

III. Results and Discussion

In both the *in vitro* and *ex vivo* evaluations, the two device designs released VAN levels sufficient to prevent adhesion of *S. aureus* to implant materials. During the *in vitro* evaluations for US-triggered VAN release (Figure 2), the PLA membrane on the 1-hole design appeared to be more stable and responsive to insonation than with the 2-hole design.

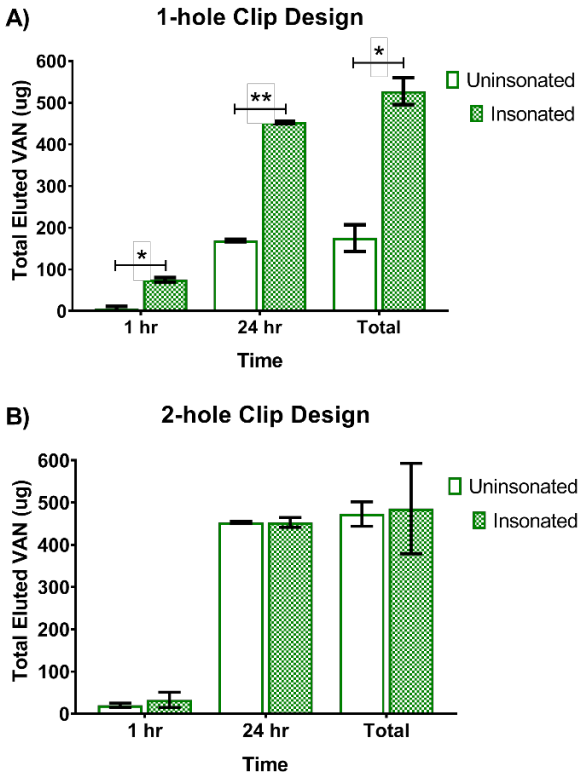

Fig. 2. *In vitro* characterization of VAN release from 1-hole and 2-hole PEEK clip designs, represented as average ± SD. A) Elution of VAN from the 1-hole clip, *p = 0.0002, **p < 0.0001. B) Elution of VAN from the 2-hole clip.

Background VAN release from the PLA-coated, uninsonated 1-hole clip was 5.43 ± 5.77 µg after submersion for 1 hour, while the 2-hole clip released 20.13 ± 5.00 µg over the same time (p = 0.015). This trend was also observed at 24 hours, where the uninsonated 1-hole clip released significantly

less VAN than the uninsonated 2-hole clip (169.41 ± 2.89 µg vs. 452.84 ± 2.89 µg, $p < 0.0001$). Additionally, there was no significant difference between the insonated and uninsonated 2-hole clips at any of the time points ($p > 0.83$, Fig. 2B).

On the other hand, the 1-hole clip exhibited markedly increased VAN release with time ($p < 0.0001$, Fig. 2A). At 1 hour post-insonation, 74.66 ± 5.77 µg of VAN was released ($p = 0.0023$ compared to uninsonated). The total cumulative VAN release from the insonated 1-hole clips was 527.50 ± 32.27 µg ($p < 0.0001$ compared to uninsonated). Interestingly, there was no significant difference in cumulative US-triggered VAN release between the 1-hole and the 2-hole design (527.50 ± 32.27 µg for 1-hole vs. 485.75 ± 107.04 µg for 2-hole, $p = 0.28$). Therefore, we continued to evaluate both the one-hole and two-hole clip designs in the *ex vivo* experiments.

Both clip designs exhibited background release of VAN consistent with levels that would be considered antimicrobial. Based on our findings in these experiments, more tuning of the coating process is necessary to improve consistency of innate and US-triggered VAN release.

In the *ex vivo* study, infected sites showed up to 10-fold reduction in bacterial colonization following US-triggered VAN release, while uninsonated sites exhibited at most a 2-fold reduction in bacterial colonization (data not shown). Negative controls indicated no surgical site contamination, and positive controls confirmed that the added *S. aureus* survived in the wound environment. A representative US image of an implanted clip is shown in Figure 3. VAN release in the rabbit model was dependent on both design and time (Figure 4). There was significantly greater US-triggered total VAN release from the 1-hole clip design than from the 2-hole design (7420 ± 2992 µg vs. 3500 ± 954 µg, $p < 0.0001$). Additionally, at 48 hours, there was significantly greater VAN release from the insonated clips compared to the uninsonated clips for both the 1-hole (Fig. 4A) and the 2-hole (Fig. 4B) clip design ($p < 0.04$).

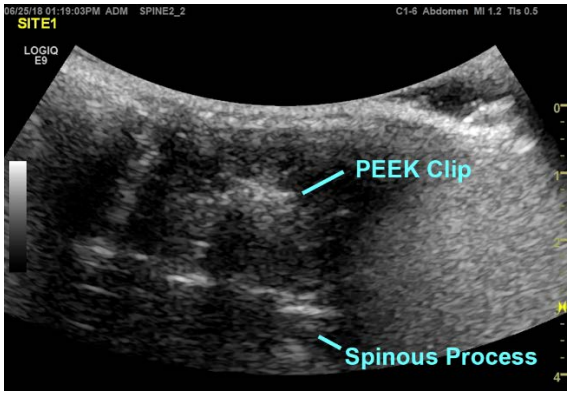

Fig. 3. Representative US image of PEEK clip implanted in the *ex vivo* rabbit spine model.

Additional studies in a living animal spinal surgery model will be necessary to fully characterize the antibiotic release and subsequent bacterial eradication with these devices. Studies are also ongoing to refine the PLA coating process, as the PLA membrane thickness and susceptibility to US-triggered rupture for release are tunable [13, 14]. Variations to the concentration of the PLA solution as well as the number of coating layers are being investigated. Study limitations include a limited sample size *in vitro* and *ex vivo*, lack of fluid flow during the VAN elution experiments resulting in saturated solutions of VAN, as well as the variability in applying and determining coating thicknesses. Finally, complete independence between sites in the *ex vivo* model was assumed but cannot be guaranteed. Overall, VAN release was visibly achieved from both device designs. We found that PLA coating of the PEEK devices is susceptible to US disruption both *in vitro* and within tissue *ex vivo*, with up to 7.4 mg of VAN delivered within 48 hr. Our result was a viable prototype (i.e., the 1-hole clip) for US mediated localized prophylactic delivery to the spinal surgical site.

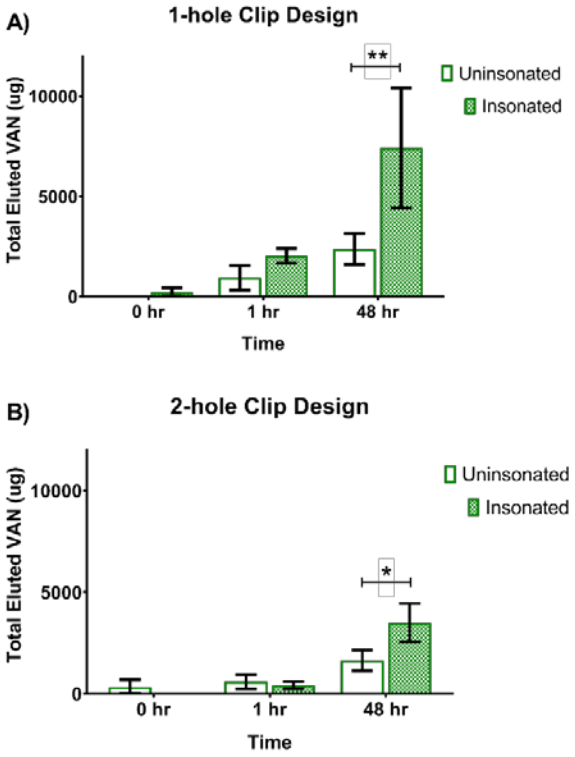

Fig. 4. *Ex vivo* characterization of VAN release from 1-hole and 2-hole PEEK clip designs, represented as average ± SD. A) Elution of VAN from the 1-hole clip, $**p < 0.0001$. B) Elution of VAN from the 2-hole clip, $*p = 0.03$.

IV. Conclusion

Existing methods for preventing infection following spinal fusion surgery are only partially successful. To combat this clinical problem, we have designed an US-activated drug release system that can release prophylactic antibiotics. The feasibility of our US-sensitive antimicrobial platform for delivering a bolus of antibiotics to a spinal wound site has been demonstrated in tissue *ex vivo*, representing an important proof of principle for continued development of this drug delivery system.

Acknowledgments

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REFERENCES

[1] S.M. Kurtz et al., "Infection risk for primary and revision instrumented lumbar spine fusion in the Medicare population," J. Neurosurg. Spine, vol. 17(4), pp. 342–347, October 2012.

[2] K.R. O'Neill et al., "Reduced surgical site infections in patients undergoing posterior spinal stabilization of traumatic injuries using vancomycin powder," Spine J., vol. 11(7), pp. 641-646, July 2011.

[3] R.A. Brady, J.G. Leid, J.H. Calhoun, J.W. Costerton, M.E. Shirtliff, "Osteomyelitis and the role of biofilms in chronic infection," FEMS Immunol. Med. Microbiol., vol. 52(1), pp. 13-22, January 2008.

[4] S.M. Kurtz and J.N. Devine, "PEEK biomaterials in trauma, orthopedic, and spinal implants," Biomaterials, vol. 28(32), pp. 4845–4869, November 2007.

[5] H.A. Crosby, J. Kwiecinski, A.R. Horswill, "Staphylococcus aureus aggregation and coagulation mechanisms, and their function in host-pathogen interactions," Adv. Appl. Microbiol., vol. 96, pp. 1-41, August 2016.

[6] S. Dastgheyb, J. Parvizi, I.M. Shapiro, N.J. Hickok, M. Otto, "Effect of biofilms on recalcitrance of staphylococcal joint infection to antibiotic treatment," J. Infectious Diseases, vol. 211(4), pp. 641-650, February 2015.

[7] S.S. Dastgheyb et al., "Staphylococcal persistence due to biofilm formation in synovial fluid containing prophylactic cefazolin," Antimicrob. Agents Chemotherapy, vol. 59(4), pp. 2122-2128, March 2015.

[8] J.W. Costerton, P.S. Stewart, E.P. Greenberg, "Bacterial biofilms: a common cause of persistent infections," Science, vol. 284(5418), pp. 1318-1322, May 1999.

[9] L.J. Delaney et al., "Ultrasound-triggered antibiotic release from PEEK clips to prevent spinal fusion infection: initial evaluations," Acta Biomaterialia, vol. 93, pp. 12-24, July 2019.

[10] C. Ketonis, S. Barr, I.M. Shapiro, J. Parvizi, C.S. Adams, and N.J. Hickok, "Antibacterialactivity of bone allografts: comparison of a new vancomycin-tethered allograft with allograft loaded with adsorbed vancomycin," Bone, vol. 48(3), pp. 631-638, March 2011.

[11] V. Antoci et al., "The inhibition of Staphylococcus epidermidis biofilm formation by vancomycin-modified titanium alloy and implications for the treatment of periprosthetic infection," Biomat., vol. 29(35), pp. 4684-4690, December 2008.

[12] A.W. Bauer, W.M.M. Kirby, J.C. Sherris, M. Turck, "Antibiotic susceptibility testing by a standardized single disk method," Am. J. Clin. Pathol., vol. 45(4_ts), pp. 493-496, April 1966.

[13] H.-W. Fang et al., "Dip coating assisted polylactic acid deposition on steel surface: film thickness affected by drag force and gravity," Mater. Lett., vol. 62(21), pp. 3739-3741, August 2008.

[14] A. Vital, M. Vayer, T. Tillocher, R. Dussart, M. Boufnichel, C. Sinturel, "Morphology control in thin films of PS:PLA homopolymer blends by dip-coating deposition," Appl. Surface Sci., vol. 393, pp. 127-133, January 2017.