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Abstract

Post-operative infection is a catastrophic complication of spinal fusion surgery, with up to 10% occurrence, and existing preventative measures (i.e., peri-operative antibiotics) are only partially successful. To combat this clinical problem, we have designed a drug delivery system around polyetheretherketone (PEEK) clips to be used for prophylactic post-surgical release of antibiotics upon application of ultrasound. The overall hypothesis is that antimicrobial release from this system will aggressively combat post-surgical bacterial survival. This study investigated a set of acoustic parameters optimized for *in vitro* ultrasound-triggered coating rupture and subsequent release of encapsulated prophylactic antibiotics. We determined that a transducer frequency of 1.7 MHz produced the most consistent burst release, and that at this frequency a pulse repetition frequency of 6.4 kHz and acoustic output power of 100% (3.41 MPa) produced the greatest release, representing an important proof of principle and the basis for continued development of this novel drug delivery system.

Key words: ultrasound-triggered release, polyetheretherketone PEEK, infection

Introduction

Bacterial infection following spinal fusion surgery is a major clinical concern, with up to 10% of patients developing post-operative infection despite aggressive peri-operative antibiotic treatments (Dastgheyb, et al. 2015, Emohare, et al. 2014, Kurtz, et al. 2012, Molinari, et al. 2012). The current standard of care for preventing post-operative infection in spinal surgery is inclusion of 1-2 g of powdered vancomycin (VAN) into the surgical site during wound closure with the intention of achieving post-surgical sterilization (O'Neill, et al. 2011). However, the outcomes of this prophylactic procedure are mixed, due to the presence of wound drains that deplete the VAN at the surgical site within the first 48 hours. Additionally, VAN is active against Gram-positive bacteria (i.e., *Staphylococci*), but Gram-negative bacteria may be present in the wound and develop into an infection. Left untreated, these remaining microbes can aggregate and form a biofilm on the implant surface, significantly complicating recovery (Hall-Stoodley, et al. 2004, LuTheryn, et al. 2019). In fact, biofilm formation is associated with up to 90% of chronic wound infections (Bjarnsholt 2013, LuTheryn, et al. 2019), and can lead to antibiotic resistance and recalcitrant infection (LuTheryn, et al. 2019, Wolcott, et al. 2008, Delaney, et al. 2019). Once this type of infection develops, treatment often requires reopening the surgical site, and in extreme cases removal of the bacterial-biofilm coated spinal fusion hardware followed by additional revision surgeries (Collins, et al. 2008, Mok, et al. 2009). With only partially effective peri-operative antibiotic treatments in spinal fusion surgery, the cost and disability due to post-operative infection will continue to rise (Collins, et al. 2008, Emohare, et al. 2014, Kurtz, et al. 2012, Mok, et al. 2009). Therefore, we aim to develop a more effective means to prevent infection to help address this clinical problem.

We hypothesize that supplementing the original supra-therapeutic concentrations of prophylactic antibiotics at the spinal fusion hardware site at a time when antibiotic concentrations are waning will lower the post-operative infection rates. In order to effectively combat post-operative infection, and at a molecular level, the formation of biofilms (Costerton, et al. 1999, Crosby, et al. 2016, Dastgheyb, et al. 2014, Rochford, et al. 2014), these new prophylactic treatment modalities should eradicate pathogens prior to adherence to the spinal hardware. Multiple studies have shown that bacteria adherence and subsequent biofilm formation, which is facilitated on the hardware surface, causes marked changes in bacterial antibiotic sensitivity due, at least in part, to alterations in gene expression as well as decreased metabolism (Costerton, et al. 1999, Crosby, et al. 2016, Dastgheyb, et al. 2014, Dastgheyb, et al. 2015, LuTheryn, et al. 2019). Therefore, we aim to target any pathogens remaining in the wound site in the post-operative period with our ultrasound-triggered prophylactic delivery device.

To evaluate our hypothesis, we have developed a customized polyetheretherketone (PEEK) clip that is incorporated into existing spinal fusion hardware by clipping onto the spinal fusion rod (Delaney, et al. 2019). This clip is designed to contain a reservoir for drug-loading, which is then sealed with polylactic acid (PLA) to serve as a non-eluting vehicle. Application of ultrasound (US) to the thin PLA membrane that seals the drug-loaded reservoir in the clip will cause this thin membrane to rupture, thus triggering a local, bolus release of supra-therapeutic levels of combinations of prophylactic antibiotics to the surgical site. A representative diagram of this delivery system is shown in Figure 1.

Targeted US application can cause mechanical disruption of drug-containing micelles and micro- or nano-particles for drug delivery applications (Postema, et al. 2004, Prentice, et al. 2005, Sirsi and Borden 2014, Tanbour, et al. 2016, Zhang, et al. 2016), including those with polymeric

shells (Bevan, et al. 2007, Eisenbrey, et al. 2010, Jablonowski, et al. 2018, Jablonowski, et al. 2017), and we intend to harness this technique for US-triggered drug delivery from our drug delivery system. One of the major advantages provided by this drug delivery system is that the release of antibiotics can be triggered and achieved at the discretion of the surgeon, without requiring additional surgery or invasive intervention. Additionally, application of US has been demonstrated to permeabilize biofilms that have formed on implant surfaces (Dong, et al. 2018, Harpaz 2000, Hu, et al. 2018), which is an added benefit of our delivery mechanism.

The purpose of this study was to determine the optimal acoustic parameters (transducer frequency, pulse repetition frequency (PRF), and acoustic output (AO) power) necessary for the *in vitro* destruction of a polymeric membrane surrounding a novel, prophylactic-containing, spinal PEEK-based clip to achieve complete release from within the device. The long-term goal is to achieve US-triggered release of antibiotics from within this spinal hardware to prevent post-surgical infection.

Materials and Methods

Device Design

C-shaped PEEK clips (1 cm diameter x 1 cm depth x 1 cm height) capable of clipping onto standard spinal fusion hardware, specifically the titanium rods used to stabilize the vertebrae, were designed with an internal drug reservoir (0.785 cm^3) to house clinically-relevant amounts of deliverable bioactive materials for triggered release, as described previously (Basgul, et al. 2018, Delaney, et al. 2019). Briefly, spacer designs were drafted using Solidworks 2016 software (Dassault Systemes, Vélizy-Villacoublay, France) and the Simplify3D slicing program (Simplify3D, Cincinnati, OH), then the PEEK clips were 3D printed by our collaborators at Drexel

University using a PEEKMed filament on an Indmatec HPP 155/Gen 2 3D printer (Apium Additive Technologies, Karlsruhe, Germany). The outer-facing surface of the clip was printed with a circular opening 4 mm in diameter (cf., Fig. 1A) for loading the bioactive material into the reservoir. The internal reservoir volume was measured with microCT imaging (Scanco μCT 80, Scanco Medical, Brüttisellen, Switzerland).

Device Loading and Preparation

Methylene blue (MeB; Sigma Aldrich, St. Louis, MO), an intense dye that is widely used to visualize drug release (Bikram, et al. 2007, Satarkar and Hilt 2008, Sutani, et al. 2002, Wu, et al. 1996), was used to visualize structural integrity of the membrane before and after insonation, as well as to clearly assess the degree of US-triggered release. PEEK clips were loaded until filled (approximately 200 μL) with a slurry of MeB, and then frozen at -20º C overnight to solidify the contents for coating with the thin PLA membrane. Loaded clips were coated by submersion and withdrawal in a solution of 1.5 g Resomer Select 100 DL 7E PLA (Evonik Biomaterials, Essen, Germany) in 30 mL chloroform (Fisher Scientific, Hampton, NH) and dried to create a thin coating on the spacer surface, encapsulating the MeB within the clip reservoir. All clips were dried under continuous air-flow at room temperature for 24 hours. This coating process was repeated four times to create a stable coating across the reservoir. Coated, loaded clips were kept at -20º C until used for testing.

Determination of US Parameters for Triggered Release

Transducer frequency, pulse repetition frequency (PRF), and acoustic output (AO) power were the three parameters sequentially evaluated in this study. Coated clips were submerged in

water to mimic the biological environment, and allowed to rest for 5 minutes to ensure no leaks from the PLA coating. Submerged clips were then insonated for 10 minutes using a Logiq E9 clinical US scanner (GE Healthcare, Waukesha, WI) equipped with a C1-6 curvilinear probe, using grayscale imaging (4.0 MHz) to establish the position of the spacer, and then power Doppler imaging (MI 1.2) to induce cavitation and rupture of the PLA coating for MeB release. The probe was positioned directly over the spacer, and held there with a clamp for the duration of the scanning, with a layer of gel between the transducer and water bag to best approximate clinical conditions.

We hypothesized that selecting a value at the lower end, middle, and higher end of the range for each of the acoustic parameters evaluated in this study would provide a comprehensive evaluation of this parameter space (Shi, et al. 2006). To evaluate this hypothesis, we followed a step-wise procedure to determine the optimal parameter value to induce membrane rupture and subsequent dye release starting with evaluation of transducer frequency, then holding the determined optimal frequency while evaluating PRF, and finally holding both optimal frequency and PRF while evaluating AO power. Initially, transducer frequency was varied, while holding the preset PRF of 1.1 kHz and 100% AO power.

Transducer frequencies evaluated were 1.7, 2.5, and 3.6 MHz, while AO energy was held at 100%. The frequency that resulted in the most consistent membrane rupture and subsequent MeB release was chosen as optimal. Once the optimal transducer frequency was chosen, PRF was evaluated at 0.1, 3.5, and 6.4 kHz at 100% AO power. Finally, the optimal transducer frequency and PRF were held while AO power was varied (30, 60, and 100%). Peak-to-peak pressures of these three AO power settings were measured with a 0.5 mm needle hydrophone (Precision Acoustics, Dorchester, UK). Briefly, the hydrophone was calibrated and placed into the US beam

at each of the study settings, and pressure measurements were taken in triplicate. Additionally, uninsonated negative controls (designated 0% AO power) were evaluated for any passive leakage during the duration of testing. Resulting MeB release was evaluated qualitatively against known MeB concentration standards immediately following insonation and again 24 hours after insonation, as appropriate to this specific study. Briefly, the degree of blue dye within the water bath at each timepoint was compared against solutions with known MeB concentrations relevant to the loading conditions in this study. The overall amount of MeB released was then calculated from the volume of the water bath and the matching MeB concentration. Clips were also visually inspected for PLA membrane rupture 24 hours after insonation.

Statistical Analysis

Results were collected in triplicate (n = 3 for each evaluated US parameter plus negative control; n = 21 total). Error represents standard error about the mean (SEAM). Statistical analysis was performed with GraphPad Prism 7 (GraphPad, La Jolla, CA) using a one-way ANOVA analysis to determine significance between groups (for $\alpha < 0.05$), with Bonferroni correction for multiple comparisons and Tukey's multiple comparison post-test when appropriate.

Results and Discussion

For the purposes of this experiment, a successful device would retain the encapsulated material until insonated, upon which the encapsulating membrane would rupture causing a burst release of the encapsulated material. Therefore, we are evaluating the acoustic parameters described in this study to determine the settings that most consistently meet these criteria for a successful targeted release. In all cases, the PLA membrane was successfully ruptured by

application of US. A representative collage of the scanning setup and resulting MeB release is shown in Figure 2. Panel A shows the overall setup before the transducer is activated, while Panels B and C show the MeB release as a result of US-triggered rupture of the PLA membrane, visualized as a blue stream emerging from the reservoir opening. Panel D shows the complete emptying of the encapsulated MeB from within the clip several hours after insonation. Additionally, representative images of the US scanning parameters are shown in Figure 3, where the standard B-mode image (Figure 3A) is used for positioning the transducer, and then power Doppler (Figure 3B) is activated to induce PLA membrane rupture. The orange areas within the power Doppler box correspond to imaging artifact, as there is no blood flow occurring within the sample. The power Doppler scanning parameters were modified as described previously to determine the optimal parameters for membrane rupture and MeB release. Finally, before and after photos of a MeB-loaded, PLA-coated device are shown in Figure 4, demonstrating the successful rupture of the PLA membrane to allow for release of the encapsulated MeB from within the clip.

Determination of Optimal Transducer Frequency

All three frequencies evaluated resulted in PLA membrane rupture and subsequent MeB release. Results of US-triggered release based on transducer frequency are summarized in Figure 5. There was no significant difference in the immediate burst release from the PEEK clips at the three transducer frequencies evaluated ($p = 0.48$), with 1.7 MHz producing 33.8 ± 7.8% release (0.23 ± 0.05 mg/mL), 2.5 MHz producing 31.3 ± 6.3% (0.21 ± 0.04 mg/mL), and 3.6 MHz producing 42.5 ± 5.0% (0.28 ± 0.03 mg/mL). After 24 hours, there was still no significant difference in MeB release between the transducer frequencies evaluated in this study ($p = 0.27$). The 1.7 MHz setting resulted in a cumulative release of 72.5 ± 17.5% (0.48 ± 0.12 mg/mL), while

2.5 MHz yielded 92.5 ± 10.9% cumulative release (0.62 ± 0.07 mg/mL) and 3.6 MHz produced 102.5 ± 2.5% cumulative MeB release (0.68 ± 0.02 mg/mL). Due to the nature of the qualitative MeB release measurements and the intensity of the MeB stain, it is not unexpected to observe a cumulative MeB release greater than 100%, and we believe that our observed results are within reason given the standard deviation of the reported average.

Ultrasound intensity lost to attenuation in tissues increase as the frequency of the ultrasound beam increases. Generally, the attenuation coefficient is 0.5 to 1 dB/cm traveled in the medium per MHz of transmit frequency (Bushberg, et al. 1994). Therefore, by selecting the lowest transmit frequency that allows for membrane rupture, we can effectively reduce the amount of signal attenuation in the surrounding tissues. Additionally, studies have shown that rupture of membranes is improved at lower US frequencies (Cohen-Levi, et al. 2000, Schroeder, et al. 2007). Considering these physical phenomena, coupled with the finding that there were no significant differences in immediate burst release between the three evaluated transducer frequencies ($p > 0.99$), we selected 1.7 MHz as the optimal frequency for burst release from these PLA-coated PEEK clips to account for signal attenuation in future *in vivo* applications.

Determination of Optimal PRF

The frequency of 1.7 MHz was maintained, while the PRF was varied to further optimize US parameters. Similar to the frequency experiments, all three PRFs evaluated resulted in membrane rupture and MeB release. Results of US-triggered release based on pulse repetition frequency are shown in Figure 6. However, unlike the transducer frequencies, there was a significant difference in resulting immediate burst release of MeB as a result of varying the PRF ($p = 0.021$). A PRF of 0.1 kHz resulted in significantly less immediate burst release of MeB (17.5

±1.3% corresponding to 0.12 ± 0.01 mg/mL) than 6.4 kHz (36.3 ± 5.4% corresponding to 0.24 ± 0.04 mg/mL, p = 0.029). There was no significant difference in immediate burst release between 6.4 kHz and 3.5 kHz (32.5 ± 2.5% corresponding to 0.22 ± 0.02 mg/mL, p > 0.99), and the difference between 0.1 kHz and 3.5 kHz was trending toward significance (p = 0.07).

After 24 hours, however, no significant differences in cumulative MeB release remained between the three PRFs evaluated in this study (p = 0.06). The 0.1 kHz setting resulted in a cumulative release of 66.0 ± 10.0% (0.43 ± 0.06 mg/mL), while 3.5 kHz yielded 75.0 ± 8.7% cumulative release (0.50 ± 0.06 mg/mL) and 6.4 kHz produced 112.0 ± 19.1% cumulative MeB release (0.66 ± 0.04 mg/mL). Again, given the standard deviation and the nature of these experiments, an average cumulative release over 100% is not unexpected. One limitation to this study is the intensity of MeB at increased concentrations, as we found that this potent dye resulted in solution saturation at the 24 hour observation point. This may have clouded the results at this second time point, hiding any potential significant differences in cumulative release from within the clips. Therefore, we place greater influence on the immediate burst release results in determining the optimal acoustic parameters, as the differences in MeB concentration are more distinguishable at this time point. As such, we were able to rule out the PRF setting of 0.1 kHz as ineffective for membrane rupture.

We did not observe any significant difference in immediate burst release between 3.5 and 6.4 kHz, suggesting that, over a certain threshold, the frequency of pulses does not have a significant impact on membrane rupture. Similarly, others have found that drug release profiles from liposomes were similar for continuous and pulsed low frequency US, and that exposure time is a more critical factor (Schroeder, et al. 2007). While there were no significant differences in burst or cumulative release between the PRF settings of 3.5 and 6.4 kHz, there was greater overall

MeB release from the clips insonated with a PRF of 6.4 kHz. Additionally, since insonation of the spinal fusion clip does not require the US wave to penetrate deep into tissue, but only through thin skeletal muscle tissues of the back, the PRF in our system is not significantly limited by ambiguity of echoed signals (Bushberg, et al. 1994). Therefore, we selected 6.4 kHz as the optimal PRF for membrane rupture for the parameter space considered in this study.

Determination of Optimal Acoustic Output Power

Finally, AO power was evaluated, while transducer frequency (1.7 MHz) and PRF (6.4 kHz) were maintained. AO powers tested were 30, 60, and 100%, which corresponded to peak-to-peak pressures of 2.33 ± 0.03, 2.72 ± 0.04, and 3.41 ± 0.01 MPa, respectively. Additionally, uninsonated controls (0% AO power, 0.00 MPa peak-to-peak pressure) were evaluated to determine any passive leakage from the membrane over the 24 hour incubation period. The results of these experiments are presented in Figure 7. Uninsonated controls exhibited only 1.3 ± 1.2% (0.01 ± 0.01 mg/mL) immediate release ($p = 0.031$ compared to 60% AO power and $p = 0.007$ compared to 100% AO power), and 6.8 ± 2.8% (0.05 ± 0.02 mg/mL) cumulative release ($p = 0.021$ compared to 30% AO power, $p = 0.006$ compared to 60% AO power, and $p = 0.002$ compared to 100% AO power) confirming US-triggered release in the insonated samples as opposed to just passive leakage. Some minor background release was observed upon submersion of some the clips in water, suggesting that the PLA membrane is not consistently stable under simulated biological conditions. Currently, we are investigating methods to improve the stability of the PLA coating membrane, including 3D-printed PLA sheets and spray application.

There was no significant difference in immediate burst release of MeB between the three AO powers evaluated, but the results were trending toward significance ($p = 0.07$). Specifically,

30% AO resulted in 12.5 ± 6.6% (0.08 ± 0.04 mg/mL) release, 60% AO produced 27.5 ± 6.6% (0.18 ± 0.04 mg/mL), and 100% AO yielded 35.0 ± 2.5% (0.23 ± 0.02 mg/mL) immediate burst release of MeB in response to insonation. While 30% AO power appears to have produced much less immediate MeB release than 100% AO power, these results were only trending toward significance ($p = 0.08$). After 24 hours, there remained no significant difference in cumulative MeB release from the insonated clips ($p = 0.39$), again likely due to the intensity and solution saturation of MeB at higher concentrations. Cumulative MeB release at 30% AO power was 75.0 ± 21.7% (0.50 ± 0.14 mg/mL), while 60% AO power resulted in 90.0 ± 8.7% (0.60 ± 0.06 mg/mL) and 100% AO power yielded 103.5 ± 0.9% (0.69 ± 0.01 mg/mL) cumulative release.

Interestingly, there was no significant difference in immediate burst release between 0% AO power and 30% AO power ($p = 0.85$), suggesting that 30% was not strong enough to completely rupture the PLA membrane for effective immediate burst release. However, after 24 hours, there was significantly greater cumulative MeB release from the sample insonated at 30% AO power compared to the 0% control ($p = 0.021$), suggesting that this low power insonation induced some damage to the PLA membrane that allowed for long-term slow release of the encapsulated MeB, just not enough to cause catastrophic membrane rupture and significant burst release immediately upon insonation. While slow elution is often used to treat establishing/established infection, the burst release is more desirable as it will sterilize the area around the implant and, due to its supra-therapeutic levels, not foster the possibility of acquisition of resistance (Dastgheyb, et al. 2015, Delaney, et al. 2019, Lewis 2008). Additionally, the temporary absence of antibiotic pressure followed by a secondary supra-therapeutic dose may help eradicate any organisms that were less susceptible to the primary treatment due to a persister phenotype (Costerton, et al. 1999, Dastgheyb, et al. 2015, Delaney, et al. 2019, Lewis 2008).

While there were no significant differences in MeB release between 60% and 100% AO power, we observed more consistent membrane rupture and greater overall MeB release from the samples insonated with 100% AO power. Moreover, the use of 100% will better counteract attenuation of the US beam in human tissue, providing a signal that is still strong enough to induce membrane rupture upon reaching the clip in its anatomical position. Hence, 100% AO power was chosen as the optimal output power within this parameter space.

Although the study findings are encouraging, several limitations must be discussed. An important limitation to this study was the small sample size. We made approximately 6 loaded, and hand-coated clips for each testing parameter, but some of these leaked prematurely or had other fabrication defects. As a result, we were able to get consistent behavior from only 3 clips per group. However, this still represents a large enough sample size for statistical comparisons, as we consider it appropriate for this proof of concept study. As previously mentioned, we found that the handcrafted PLA coating efficiency was inconsistent across PEEK clip samples, with some leaking immediately upon introduction to water and some not rupturing at all in response to insonation. As such, future work will include developing methods to standardize the PLA coating for more consistent sealing of the reservoir, including investigation of 3D printed capping lids for the reservoir and spray coating of the PLA layer. However, we have previously demonstrated the long-term (7 day) stability of the PLA layer (Delaney, et al. 2019), and are confident that the bulk of the encapsulated material would remain contained within the device until insonation. Remaining PLA material will be hydrolyzed into lactic acid over time and naturally secreted. Overall, there was no significant difference in cumulative release after 24 hours for any of the evaluated acoustic parameters ($p > 0.50$), likely due to the saturation effects of MeB dye in solution as discussed previously. Ongoing work includes the use of antibiotics or other bioactive compounds that are

more readily quantified by more precise means and are not subject to visual saturation. Nonetheless, the MeB allowed for clear visual confirmation of membrane rupture and provided important information about the PLA membrane stability, serving as an appropriate model in these proof of concept experiments. Finally, we recognize that the acoustic parameters evaluated in this study represent only a subset of the totality for frequency, PRF, and AO power. The test values chosen for each parameter were based on the settings available on the Logiq E9, and represent the lowest, highest, and middle points on the ranges of available settings. Additional studies are needed to more completely optimize the acoustic parameters for PLA coating rupture and subsequent release, including manipulation of duty cycle, pulse shape, insonation time, and therapy times. Nonetheless, these results represent a proof of principle and basis for these continued studies, especially as we prepare to move into an *in vivo* model. In summary, a transducer frequency of 1.7 MHz, a PRF of 6.4 kHz, and 100% AO power was determined to be optimal for US-triggered release for the parameter space studied here. These settings ultimately resulted in a total peak-to-peak pressure of 3.41 ± 0.01 MPa, which is well within the acceptable limits for clinical US scanners (Barnett, et al. 2000, Duck and Henderson 1998).

Conclusions

Post-operative infection is a catastrophic and far too frequent complication of spinal fusion surgery (occurrence rate of up to 10%), with existing preventative measures being only partially successful. To combat this clinical problem, we have designed a drug release system to be used in spinal surgeries for prophylactic post-surgical release of combinations of antibiotics (i.e., both Gram-positive and Gram-negative targets) upon US application 5-7 days after surgery or at the surgeon's discretion. We believe that this system will aggressively combat the survival of bacteria

contaminating the surgical site, while also allowing versatility in loading capacity (i.e. combination of Gram-positive and Gram-negative antibiotics), providing a tailored solution to the clinical problem that we feel is superior to controlled, prolonged release systems. This study represents a proof of principle and basis for continued development of this US-triggered drug delivery system. We have successfully determined a set of primary US scanning parameters (1.7 MHz transmit frequency, 6.4 kHz PRF, and 100% AO power, corresponding to 3.41 MPa peak-to-peak pressure) that are appropriate for US-triggered rupture of the encapsulating PLA membrane and the subsequent release of encapsulated bioagents. While minimal background release was observed from some of the devices, overall we have demonstrated significant burst release of encapsulated materials upon insonation compared to uninsonated controls. These results serve as an important initial step towards deployment of this prophylactic device.

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Figure Captions List

Figure 1: Representation of novel spinal hardware. A) Drawing of loaded, coated PEEK clip. B) US-triggered release from anatomical positioning on spinal fusion hardware.

Figure 2: Representative images of the ultrasound scanning setup for triggering membrane rupture and MeB release. A) Setup as a whole prior to insonation, showing the clip submerged in water and positioned under the transducer. B) Image of initial PLA membrane rupture and resulting MeB release. C) US-triggered MeB release from ruptured PLA membrane, with fluid streaming from within the clip. D) Resulting complete MeB release from within the clip following insonation.

Figure 3: Ultrasound imaging of MeB-loaded, PLA-coated PEEK coated. A) Grayscale image of spacer. B) Power Doppler scanning of spacer.

Figure 4: Representative images of MeB-loaded, PLA-coated PEEK spacer. A) Spacer prior to insonation with intact PLA coating. B) Spacer after insonation, with fully ruptured PLA coating.

Figure 5: Summarized results of US-triggered MeB release based on transducer frequency, n = 3, error bars represent standard error about the mean.

*Figure 6: Summarized results of US-triggered MeB release based on pulse repetition frequency, *p = 0.029 between 0.1 kHz and 6.4 kHz immediate burst release, n = 3, error bars represent standard error about the mean.*

*Figure 7: Summarized results of US-triggered MeB release based on acoustic output power, with 0% AO power negative controls, top*p < 0.02 for MeB cumulative release from 0% AO power being significantly less than the other 3 parameters, bottom*p < 0.03 for immediate burst release of MeB from 0% AO power compared to 60% and 100% AO powers (0% vs. 30% not significant, p =0.85) , n = 3, error bars represent standard error about the mean.*

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