Whitepaper

TITLE: **The Urgent Need for Improved Antibiotic Release Profiles for Bone and Joint Infections.**

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1. Summary:

The infection of bone and joints is a devastating complication for patients. Bone infections can be caused by trauma in the case of open fractures, progressive soft-tissue infections, or can be hematogenous. Orthopedic implants, including prosthetic joint replacements account for the largest segment of bone and joint infections. With the significant increase in planned joint replacements forecast over the next 10 years, combined with the critical need for antibiotic stewardship in the face of the global emerging threat of antibiotic resistance – the need to significantly improve methods of treating bone and joint infections is urgently required.

2. Introduction:

Infection of bone or prosthetic joints and surrounding tissue is a very serious complication. A common incident of bone infection is after surgery, and following open fractures, where pathogens are exposed to the bone tissue. Bone can also become infected by progressive soft-tissue infections (e.g., diabetic foot ulcers which have progressed to the bone), or blood borne pathogens which are more common amongst intravenous (IV) drug users. A significant orthopaedic challenge is when a joint and its adjacent tissue become infected following artificial joint arthroplasty. Periprosthetic joint infections (PJI) occur in 1% to 2% of primary arthroplasty operations (Ahmed and Haddad, 2019) and the risk of revision increases three-fold for total hip arthroplasty in cases with PJI compared to cases without PJI (Lenguerrand *et al.*, 2017) .

PJI also increases the medical costs, which are up to 24 times higher than without PJI (Zimmerli, Trampuz and Ochsner, 2004). The major cost of PJI is generated by prolonged hospitalization, multiple surgeries and prostheses, and medical supplies (Alp, Cevahir and Ersoy, 2016). Infections of both bones and periprosthetic joints are difficult to control and prone to reinfection, due to the formation of bacterial biofilms, which protect the bacteria from antibiotics and the immune system. For some bacterial species, the minimum biofilm eradication concentration (MBEC) can be 100 to 1,000 times greater than the minimal inhibitory concentration (MIC) for the same antibiotic (Argenson *et al.*, 2018), requiring much higher antibiotic concentrations for the effective eradication of bacterial biofilms (Lechner, Lewis and Bertram, 2013; Dosler and Karaaslan, 2014).

Development stages of the biofilm formation are divided into 4 phases; namely adhesion, proliferation, biofilm maturation and cellular detachment (Gbejuade, Lovering and Webb, 2015). The bacterium can adhere to the non-viable host material (e.g., necrotic bone fragments) or a foreign body material (e.g., orthopaedic implants). Gristina coined the term “race for the surface”. He argued that implanted biomaterials tend to potentiate bacteria on their surfaces so that normally friendly special or opportunistic organisms become virulent pathogens. Virulence is also enhanced because both bacteria and biomaterials interfere with host defence mechanisms (Gristina, Naylor and Myrvik, 1988).

In attempting to overcome the bacterial challenge, patients are provided with systemic antibiotics administered intravenously (IV) over several weeks. However, systemic antibiotics administered intravenously (IV) can only reach two to three times the MIC in joints and infected tissues (Roy *et al.*, 2014). Increasing the IV antibiotic dose increases the incidence of antibiotic-associated adverse events, such as hepatic and renal toxicity, and longer courses of antibiotic therapy are associated with increased rates of antibiotic resistance without increasing cure rates (Tam *et al.*, 2007; Byren *et al.*, 2009; Smirnova *et al.*, 2009; Stern *et al.*, 2010). Therefore, systemic antibiotic administration is potentially an insufficient approach to achieving local MBECs for either bone or joint infections.

To overcome the limitations of systemic antibiotic therapy in bone and joint infections, surgeons have adopted the local use of antibiotics. Common approaches include the use of intrawound antibiotic powder or solution added perioperatively into the wound site; the use of antibiotic loaded polymethylmethacrylate (PMMA) spacers; and antibiotic loaded calcium sulphate (CS) products.

This paper provides new insights into the antibiotic release profile of resorbable calcium sulphates and contrasts these findings with published results of PMMA spacers. The paper then proposes an ideal release profile which can serve as a guide to future biomaterial product research and development.

PMMA Cement

The use of a PMMA cement spacer, loaded with antibiotics represents the current gold standard of treatment in the USA (Vaishya, Chauhan and Vaish, 2013). PMMA is a non-resorbable material. Thus, a second stage procedure is required to remove the PMMA cement spacer before it becomes entrapped within host tissue, pursuant to a foreign body response. The requirement for a second surgical procedure introduces additional patient risk, such as increasing chances of further infections. The release of antibiotics from PMMA Cement has been studied by several authors. They report low amounts of the incorporated antibiotic are released from the cement (~4%), and the antibiotic which is released describes a burst effect, with ~80% in the first 24 hours (Wei *et al.*, 2022). The antibacterial effects against MRSA have been shown to last for less than three days (Duffy and Shafritz, 2011; Lee *et al.*, 2016; Hsu *et al.*, 2017; Boelch *et al.*, 2018; Wei *et al.*, 2022).

Calcium Sulphate (CS)

An alternative class of emerging product which substitutes PMMA bone cements are made from Calcium Sulphate (CS). CS has been used as bone graft substitute to fill bone defects since 1892 (Dressman, 1892). It was found that normal bone grew in the area where CS was implanted, it did not stimulate osteogenesis but it did not have any adverse effect in the adjacent tissues (Ricci *et al.*, 2008). In the current market, CS materials are mainly used in bone sites as bone void filler to prevent soft tissue growth until the bone has regenerated (Ricci *et al.*, 2008). Its mechanical property suggests it can be used as a bone void filler only, as it does not have load bearing properties. CS is a haemostatic agent (Scarano et al., 2012), it has angiogenic properties (Strocchi *et al.*, 2002) and it can act as a delivery vehicle for drugs and growth factors (Orellana, Hilt and Puleo, 2012). CS is a resorbable material and it is also used to deliver therapeutic agents in arthroplasty (Mohamed *et al.*, 2020), open fracture (Helgeson *et al.*, 2009) and chronic osteomyelitis (Shi *et al.*, 2022). CS beads are usually prepared from moulds, antibiotics powder are mixed together until a ‘doughy’ paste is created (Abosala and Ali, 2020). Different antibiotics are reported to be mixed with CS such as vancomycin (Lum and Pereira, 2018), gentamicin (Drampalos, Mohammad and Pillai, 2020), tobramycin (Kallala *et al.*, 2018) and cefazolin (Lum and Pereira, 2018).

The current market leader is Stimulan by Biocomposites (K141830). In Europe, the product is approved for use with antibiotics. In the USA, the FDA have not cleared it as an antibiotic carrier. Surgeons however regularly use CS materials supplemented with antibiotic at the point-of-use.

Recently, BoneSupport AB have received a De Novo 510k for their Cerament-G product, which contains 60% CS and 40% hydroxyapatite. The CS element acts a delivery vehicle for the antibiotic gentamicin sulphate. This is the first and only resorbable bone graft, with antibiotic elution, cleared for the US market, and the indication is for osteomyelitis only, and does not include use in trauma or periprosthetic joint infections.

Calcium sulphate-based products, however, exhibit several limiting factors for their use as a synthetic bone substitute for antibiotic delivery. CS is rapidly resorbed and has poor bone healing (Jepegnanam and Von Schroeder, 2012). In a different study, Ferguson et al. evaluated another antibiotic-loaded calcium sulphate product, Osteoset (K010532). Results suggested poor bone healing, with one study, showing complete filling of the defect in only 4.4% of cases and partial and no bone healing in 59.0% and 36.6% of cases respectively (Ferguson *et al.*, 2014). Furthermore, antibiotic elution rates from CS beads are very rapid (Moore *et al.*, 2021). This rapid drug elution rates have implications. Firstly, within the first few hours of release, following initial implantation, supraphysiological doses of the drug are released leading to high toxicity to local endogenous cells (Berry, Gurung and Easty, 1995; Yoeruek *et al.*, 2008; Braun *et al.*, 2020). Secondly, the initial burst release of the drug has limited delivery potential above the minimum inhibitory concentration (MIC) (i.e., 2-3 days rather than extended weeks). Sustained antibiotics release is required to prevent bacterial colonization and biofilm formation (Howlin *et al.*, 2015). And thirdly, after the initial drug burst there is a level 'tail' release of the antibiotic which provides the potential threat of antibiotic resistance if below MIC levels (Li and Webster, 2018).

In clinical setting, patients' complications from CS bead used for antibiotic delivery have been reported extensively (Ene *et al.*, 2021) . Kallala et al. reported three specific side effects in a large patient's case of 755, who underwent knee or hip arthroplasty revision surgery. These side effects were: prolonged wound drainage, transient hypercalcemia, and heterotopic ossification (Kallala *et al.*, 2018). Furthermore, it has been observed that the incidence of prolonged wound drainage and hypercalcemia is directly correlated to amount of CS beads implanted. Specifically, the occurrence of hypercalcemia increases with the volume of CS beads greater than 20cc (Kallala and Haddad, 2015).

CS beads have also been used in periprosthetic joint infection (PJI) as an auxiliary treatment. In a study conducted by Flierl et al., the use of CS beads appeared to have not improve the outcome of the 32 DAIR procedures of acute hematogenous or acute PJI (Flierl *et al.*, 2017).

In a study conducted by Post et al. it becomes clear the importance of extended release of vancomycin over its concentration. The study was designed to determine the eradication concentration of *Staphylococcus aureus* biofilm. Reduction in viable bacteria was observed over time at all concentration above 100mg/L, demonstrating a time-dependent correlation between vancomycin and biofilm eradication. Importantly, vancomycin activity against *S. aureus* biofilms was not concentration dependent. Increasing the concentration of vancomycin did not have a significant effect in overcoming the biofilm. These result have profound implication on what the ideal delivery profile should be for vancomycin as drug delivery vehicle (Post *et al.*, 2017).

Given the current limitations of CS beads, there is scope to explore different engineered biomaterials to be used as antibiotic carriers to tackle bone and joint infections. Any new material should be biocompatible and resorbable, to avoid a costly second procedure which introduces risks to the patient. The new material should also demonstrate Programmed Drug Release, which is the improved matching of molecule bioavailability with the biological need. The key principles taught by the authors on this subject can inform the ideal release profile to guide the development of new products.

Firstly, within the first few hours of release, following initial implantation, supraphysiological burst release doses of the drug should be avoided as this leads to high toxicity to local endogenous cells (Berry, Gurung and Easty, 1995; Yoeruek *et al.*, 2008; Braun *et al.*, 2020).

Secondly, the release in the first few hours should be above the MBEC, to take advantage of the physically disruption of biofilm caused during the surgical debridement and irrigation. Wolcott et al. identified a 24–48 h therapeutic window during which antibiotic therapy was more effective following incision (Wolcott *et al.*, 2010).

Thirdly, based on the important findings by Post et al., the importance of a prolonged release profile extending over at least four weeks is critical in the delivery of antibiotics that act on cell wall synthesis for their antimicrobial effect (Post *et al.*, 2017).

Thus, an ideal release profile should be above the MBEC for at least 48h, but below the toxic level during the initial accelerated release period, followed by a sustained and controlled release of the API over an extended period (4+ weeks) Figure 1.

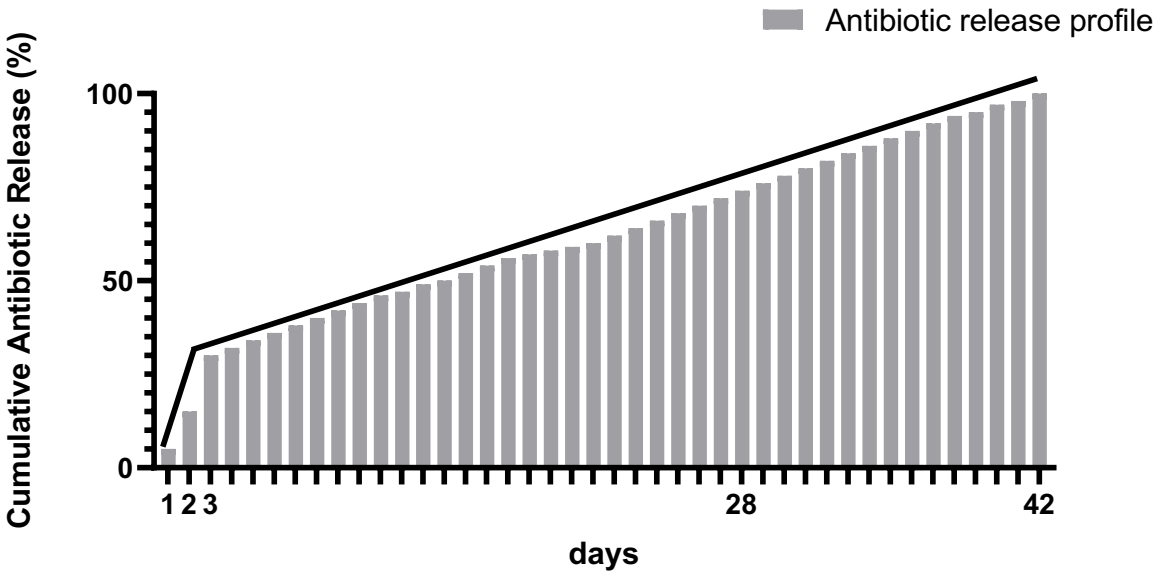

Figure 1. Ideal antibiotic release kinetics. Initial burst over 3 days (above MBEC) and sustained release over 7 weeks.

Different bioresorbable carriers have been used to treat bacterial infection in orthopaedic application, and they are covered extensively in the review by Allizond et al. (Allizond *et al.*, 2022). However, the optimal release profile (Figure 1.) of any antibiotic from a bioresorbable carrier is yet to be found.

3. Materials and Methods

3.1 Vancomycin and Gentamicin antibiotic dilution assay from CS beads

Four grams of beads were prepared as per Stimulan and Osteoset manufacture instruction, with the addition of either vancomycin or gentamicin (Gitelis and Brebach, 2002; Aiken *et al.*, 2015). Cerament was also prepared as per instruction using similar molds to fabricate the beads. During the fabrication process of Cerament product, some leakage from the syringe was noted. Once the beads set and hardened, they were placed in plastic tubes and 10 ml of PBS was added. Tubes containing beads were incubated at 37 °C. Samples were taken at chosen time points for up to 27 days. At each time point, half of the PBS was removed and replaced with equal amount of PBS to simulate mass transit of fluids *in vivo*. Two independent samples were analyzed for each CS condition.

Vancomycin concentration eluted from the beads was measured with at 280nm UV absorbance. Gentamicin concentration was measure with Fluoraldehyde™ o-Phthaldialdehyde reagent derivatization and UV absorbance at 330nm with 450nm reference wavelength. The standard curves correlation (R^2) for all antibiotics was always above > 0.99.

4. Results

Both gentamicin and vancomycin antibiotics elution profile were investigated from CS carrier beads. More than 99% of vancomycin was eluted from Stimulan beads within 6 days. More than 50% of vancomycin was released within 24 hours (21mg/cc). Gentamicin was eluted much quicker, and all the antibiotic was released within 24 hours.

Osteoset beads had a similar release profile to Stimulan for both vancomycin and gentamicin. All gentamicin was released within 24 hours, whereas all vancomycin within 9 days. Similarly, more than 50% of vancomycin was released from Osteoset beads within 24 hours (17.6 mg/cc). Vancomycin and Gentamicin from Cerament beads, also, display a very rapid burst release. All vancomycin was release from Cerament beads within 24 hours, whereas gentamicin within 6 hours. Overall, the curves from all CS products had an initial burst release and both antibiotics from all the products were releases within 24 hours for gentamicin and within 9 days for vancomycin, respectively.

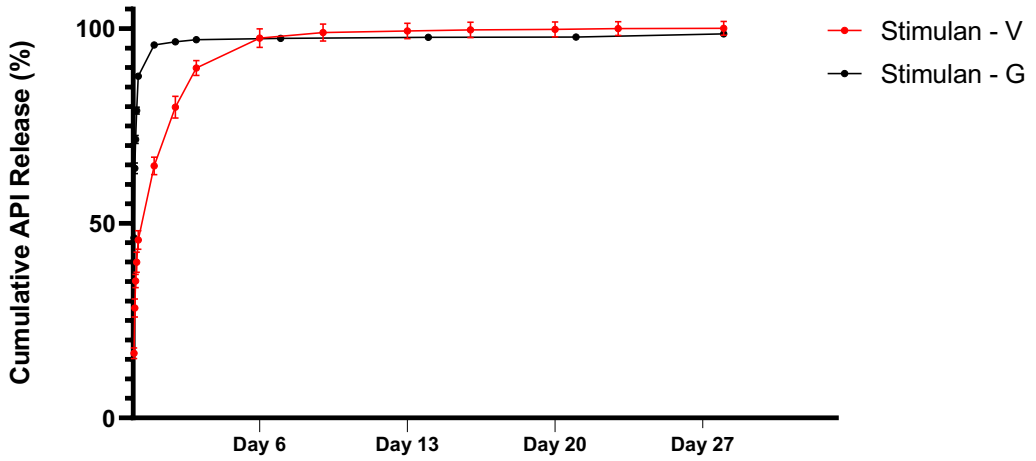

Figure *2*. **Vancomycin and Gentamicin release profile from STIMULAN.** Cumulative release curve showed that both antibiotics have an initial burst. All the loaded vancomycin is released within 6 days while all gentamicin is released within 24 hours.

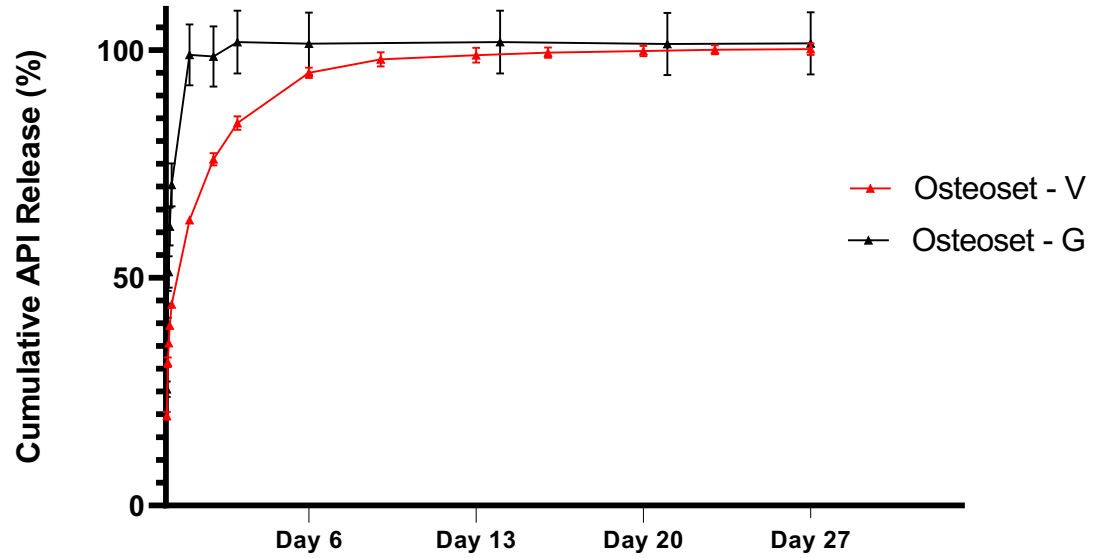

Figure *3*. **Vancomycin and Gentamicin release profile from OSTEOSET.** Cumulative release curve showed that both antibiotics have an initial burst, and all the loaded vancomycin is released within 9 days while all gentamicin is released within 24 hours.

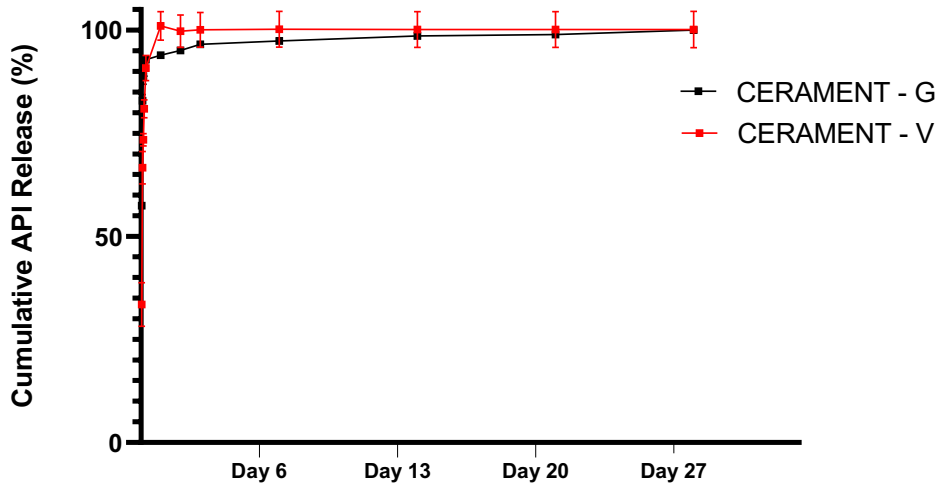

Figure *4*. **Vancomycin and Gentamicin release profile from CERAMENT.** Cumulative release curves showed that both antibiotics have an initial burst, and all the loaded vancomycin is released within 24 hours while all gentamicin is released within 6 hours.

5. Discussion

Both vancomycin and gentamicin release profile from all the CS products appear to have a large burst within the first 24 hours of elution (Figure 1-4), releasing more than 50% of the antibiotic for all conditions. These results are partially in agreement with the results from a study conducted by Aiken et. al (Aiken *et al.*, 2015). In their study, vancomycin, released from Stimulan beads, peaked after 48 hours, whereas gentamicin released from Stimulan beads peaked after 6 hours. However, the author expressed caution when comparing his results with different studies as the design of experiment could have an impact on antibiotic elution profiles (Aiken *et al.*, 2015). In our study, all the vancomycin was released from CS Stimulan beads within 9 days, with 0.7% being released between day 6 and day 9. No more eluted vancomycin was detected after 9 days as it is shown in the cumulative release graph, where a tail can be seen from day 9 until day 27. Contrarily, Aiken et al, show vancomycin been released from CS beads up to day 42 (Aiken *et al.*, 2015). In a different study involving CS beads and vancomycin, Wichelhaus et al. showed vancomycin *in-vitro* elution occurred within 10 days from CS beads (Wichelhaus *et al.*, 2001), a much shorter elution period compared to the results presented by Aiken et al (Aiken *et al.*, 2015). However, the way the beads were manufactured differed from Aiken and our fabrication method. In another study, Roberts et al. showed that all vancomycin was eluted within 10 days using the same method we have used to manufacture the CS beads. These finding are in agreement with ours, where we saw all vancomycin being eluted within 9 days. Interestingly, their results also showed that more than 50% of vancomycin was eluted within 24 hours. In their study Robert et al. further investigated the efficacy of CS vancomycin loaded beads against *E. faecalis,* and reported that, after 5 days, elution concentration was below the MIC required to inhibit bacterial growth (Roberts, McConoughey and Calhoun, 2014). These findings raise important questions whether the presence of the antibiotic eluted from CS materials at sublethal levels might promote antibiotic resistance. This effect is likely to be pronounced in the presence of biofilm, as the elution at levels at or below the MIC rather than MBEC level will clearly be sublethal for bacteria with lower metabolic activity, or otherwise provided with high tolerance due to the extracellular matrix of the

biofilm. In an *in-vivo* study, Stravinskas et al. showed that the peak elution of vancomycin is reached after 6 hours post-surgery and decrease linearly up to 2.5 day (Stravinskas *et al.*, 2019).

The initial antibiotic drug burst from all CS products that we have found in our study, is consistent with other reports of *in-vitro* antibiotics' elution (Wichelhaus *et al.*, 2001; Roberts, McConoughey and Calhoun, 2014; Aiken *et al.*, 2015). These supraphysiological level of antibiotics in the first 24 hours have the potential of killing local endogenous cells, preventing cells integration and proliferation, and eventually impairing bone regeneration.

6. Conclusion

This paper considers the performance of antibiotic delivery vehicles for the treatment of bone and joint infections. It focuses on calcium sulphates (CS) and summarises the findings of other authors on the topic. New information on the release profiles of gentamicin and vancomycin from CS and CS:HA combined products are presented. These release profiles are then contrasted with a proposed ideal release profile, and the shortcomings discussed. The alarming increase worldwide in microbial resistance crystallizes the importance of improved release profiles from biomaterials, over those discussed in this paper.

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