Mixing Method Affects the Release and Compressive Strength of High Dose ALBC
Miller, R1; McLaren, A1,2; Leon, C2; Calara, F2; +McLemore, R1,2;
+Banner Good Samaritan Orthopaedic Residency, Phoenix, AZ; 2School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ
ryan.mclemore@gmail.com

INTRODUCTION:
Orthopaedic implant infections are caused by biofilm forming microbes. Control of active orthopaedic infection relies on debridement of all biofilm and treatment of the remaining contamination with local antimicrobial delivery. Local antimicrobial delivery is intended to create antimicrobial concentrations 100-1000x the MIC (minimum inhibitory concentrations) necessary to successfully kill planktonic bacteria. A common technique for local drug delivery is the use of antimicrobial loaded bone cement (ALBC) in either beads or spacers. The “high-dose” (>10g antimicrobial/batch) formulation of ALBC is not available and must be formulated by the surgeon in the operating room. The optimal mixing method has not been determined. Data from previous studies of low-dose formulations are unlikely to apply to high dose formulations in which the volume fraction of powder is sufficient to form interconnected pores. This study questions how mixing method affects antimicrobial release and compressive strength of ALBC containing high dose antimicrobial powder.

METHODS:
10g of vancomycin hydrochloride (14 vol%) was mixed into Simplex® cement using one of three mixing methods: 1) Hand-Stirred: antimicrobial powder was ground fine then hand stirred homogeneously into the polymer powder using a spatula, prior to monomer addition. 2) Bowl-Mixed: antimicrobial powder was ground fine then mixed homogeneously into the polymer powder using a commercial mixing bowl, prior to monomer addition. 3) Dough Phase: antimicrobial powder was left in chunks (1-5 mm) and folded in during the dough phase after monomer addition. Three batches of cement were mixed for each mixing method. After mixing, cement formulations were placed in Teflon molds to form standardized test cylinders, 12 mm by 6 mm diameter (ASTM F451-08). To evaluate antimicrobial release, 5 cylinders for each batch (n=15/mixing method) were individually eluted under infinite sink conditions in 5 mL of DI water at 37°C. Total eluant exchange was performed on days 0.5,1,3,7,15, and 30. Vancomycin concentration was assayed by isocratic HPLC. Briefly, a Phenominex Prodigy 5u ODS column was used with a mobile phase of 89:2 vol/vol acetonitrile:2%TEA in water(pH3). The flow rate used was 2 mL/min. Detection was performed at 220 nm. The area under the curve (AUC) was determined for standards of known concentrations, and a standard curve was used to calculate the concentration of vancomycin present in the eluate from each cylinder. Concentration was multiplied by eluate volume, and summed over time to calculate the total amount of released antimicrobial (Mt).

For compression, three groups of 5 cylinders per batch (n=45/method) were eluted in 25 mL of DI water under infinite sink conditions at 37°C with total eluant exchange on day 0.5,1,3,7,15, and 30. Cylinders were loaded to failure at 24 mm/min on an MTS Syntech 1S load frame after 0, 1, or 30 days of elution.

Mt and compressive strength were analyzed using Repeated Measures ANOVA (RM ANOVA), with batch as a nested variable in method, and p=.05 as the level of significance. Standard normal plots of residuals of the ANOVA were examined for linearity to confirm suitability. Post-hoc comparisons employed t-tests.

RESULTS SECTION:
Over 30 days Hand Stirred ALBC delivered 7,700 µg of vancomycin, Bowl Mix ALBC delivered 11,731 µg and Dough Phase ALBC delivered 18,570 µg. RM ANOVA analysis of antibiotic delivery showed that mixing method was significant in determining delivery (p<0.001). An estimated 13% of the antimicrobial was recovered for the Hand Stirred cement, while an estimated 32% of the antimicrobial was recovered for the Dough Phase ALBC. Delivery from Dough Phase ALBC was more variable than that from the other two methods (p<0.001).

Compressive strength was 102 MPa for Hand Mix, 96 Bowl Mix and 72 for Dough Phase mixing method. RM ANOVA on compressive strength showed significance for time in elution (p<0.001), and mixing method(p<.001), but not batch (p=8.13). Dough Phase ALBC exhibited considerably more variance in release than either Bowl-Mixed or Hand-Stirred ALBC. By day 30, all samples had decreased compressive strength; 56 MPa for Hand Mix, 56 MPa for Bowl Mix and 36 MPa for Dough Phase. The Dough Phase ALBC sustained catastrophic failure, fracturing into several pieces, whereas cylinders made of ALBC from the other two methods underwent plastic deformation.

DISCUSSION:
This study investigated the effect of mixing method on elution and compressive strength of high dose ALBC. Previous studies reported an insignificant effect of mixing method on elution and compressive strength of low dose ALBC. High dose ALBC shows more variation based on mixing method, with a meaningful effect (double) on the amount of antimicrobial delivered. Dough Phase mixing of ALBC is quite different than mixing of either of the other two cements. The Dough Phase cement becomes caky, with a dusting of powder over the entire surface as it is setting, and is stiffer and harder to handle during that phase, whereas both bowl mixed and hand mixed cement are much more visually homogeneous and malleable in the dough phase.

High dose Dough Phase ALBC released more vancomycin but was weaker in compressive strength than the other two mixing methods. Hand-Stirred and Bowl-Mixed ALBC provided similar behavior over the 30 days of elution, although Bowl-Mixed trended higher than Hand-Stirred for all time-points.

It is unknown how vancomycin compares to other porogens /antimicrobial powders for release and mechanical strength in high dose ALBC.

The low mechanical strength of eluted high dose cement from all mixing methods is expected. The visible difference in failure mode in the Dough Phase cement is likely related to larger voids within the material related to this mixing method, as is the lower compressive strength.

It is unknown if the standard test cylinder size affects these data. The pore size is large compared to the radius of the cylinders for the Dough Phase ALBC. The release data for Dough phase ALBC likely represents the release behavior of beads accurately but may be over-estimated for spacers as the cylinders have a much larger surface area to volume ratio than spacers.

Based on these data, Dough Phase ALBC would be expected to deliver more vancomycin to a surgical wound than either of the other two mixing methods studied, but is more likely to lead to mechanical failure under load.

SIGNIFICANCE:
When hand mixing high dose ALBC, Hand Stirring or Bowl Mixing methods may have stronger mechanical properties for spacers, and Dough Phase mixing may deliver more antimicrobial elution from beads.