Functionalized Surfactant Increases Mechanical Strength and Gentamicin Elution from Emulsified Antimicrobial Loaded Bone Cement

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Statement of Purpose:

Water and methyl methacrylate monomer are immiscible liquids and water-in-monomer emulsions are not stable long enough for polymerization without separation of the aqueous component. Surfactant stabilizes the emulsion long enough to allow polymerization and the resultant polymethymethacrylate (PMMA) is porous. When antimicrobial solution is used for the aqueous phase of the emulsion the resultant Antimicrobial Loaded Bone Cement (ALBC) is porous with increased antimicrobial release compared to non-emulsified ALBC, however, the compressive strength of emulsified ALBC is not strong enough for implant fixation. Conjugating acryloyl chloride to the surfactant gives it the potential to crosslink the PMMA thereby increasing the strength of emulsified ALBC, possibly to an acceptable level for implant fixation. It was hypothesized- Emulsified ALBC is strengthened by functionalizing the surfactant with acrylol chloride.

Methods:

Synthesis of Functionalized Surfactant

Acryloyl chloride diluted in tetrahydofuran was added drop wise to PEG-PPO-PEG at 4°C, then 12 h at room temperature. Precipitation was performed in a 10-fold excess of diethyl ether, then it was filtered, and dried under reduced pressure. The product was dissolved in deionized water, dialyzed for 3 days against deionized water, and lyophilized leaving liquid functionalized surfactant.

Formulation of Emulsified ALBC

Emulsified ALBC formulations were made using two different surfactants, 1) PEG-PPE-PEG (S) and 2) functionalized PEG-PPO-PEG (FS) as synthesized above. Emulsions of antimicrobial solution-in-monomer was prepared by vortexing 1.5mL gentamicin solution (1gram gentamicin, New Chemic, Montvale, NJ) in deionoized water), 3.5 mL surfactant (S or FS) and 20mL monomer, resulting in the ratio of 6%-14%-80% by volume. 20 mL of the emulsion was used to polymerize 40 g PMMA powder (Simplex®P bone cement, Stryker, Mahwah NJ). Controls were prepared with 1g gentamicin powder mixed by hand into the PMMA powder before polymerization. Test cylinders, 6 mm in diameter by 12 mm in length, were made in a Teflon mold, (ASTM 451-99). The ends of the cylinders were machined flat and square.

Elution Studies

15 test cylinders from each ALBC formulation were individually eluted in 5 mL of deionized (DI) water under infinite sink conditions at 37°C. Total eluant exchange was performed at 1, 7, 15, and 30 days. Gentamicin concentrations were assayed by disc diffusion bioassay. Cumulative recovered gentamicin, M_t , was calculated.

Compression Testing

15 cylinders from each formulation of ALBC were loaded to failure in axial compression at 24mm/min, 5 before elution and 5 each after 1 and 30 days of elution as described above, using a MTS Syntech 1/s mechanical testing machine (MTS Grand Prarie MN).

Electron Microscopy

Cylinders from each ALBC formulation were split and imaged with electron microscopy before elution and after elution for 1 and 30 days.

Statistical Analysis

Repeated Measures Analysis of Variance (RM ANOVA) was used to determine the effects of formulation over time (1, 7, 15 and 30 days for elution; 0, 1 and 30 days for compression), with t-tests as post-hoc test to determine differences between groups. Two tailed paired t-tests were used to compare between cylinders of the same formulation over time. Statistics were performed using MINITAB (Minitab INC, State College, PA). Normal probability plots were used to confirm approximate normalcy of residuals.

Results:

Elution: M_t for FS-ALBC at 15 and 30 days was 1044 μg and 1207 μg; greater than S-ALBC, 468 μg and 557 μg respectively, and greater than control,153 μg and 185 μg respectively ($p=0.01$, RM ANOVA),

Compression: Formulation was a significant factor in determination of Compressive Strength ($p<0.01$, RM ANOVA). Compressive strength of FS ALBC was 73 MPa before elution and did not change with time in elution, reaching 68 MPa and 67 MPa after 1 and 30 days of elution, respectively. The compressive strength was greater than the compressive strength of S-ALBC, 60 MPa at 30 days ($p=0.006$) but less than non-emulsified control at 15 days, 72 MPa, but roughly equal at 30 days, 58 MPa ($p= 0.02, 0.289$).

Imaging: At 250 x and 2500x magnification under eSEM, many pores measuring 10 – 100 μm were seen throughout the FS-ALBC. The pores were similar in size but had many more interconnections than the pores seen in S-ALBC. Pores were rare in the control ALBC.

Conclusions:

FS-ALBC released nearly 2x faster than the gentamicin release from S-ALBC by day 7 and continued to release gentamicin 1.5x faster through day 30. Release from the control was negligible after day 15. This may be due to greater pore interconnectivity seen in FS-ALBC. The strength of the FS-ALBC was increased over the non-funtionalized ALBC and it did not degrade over time but it is still not strong enough for implant fixation (ISO 5833: 70 MPa). Further optimization of the emulsified ALBC will be needed before implant fixation can benefit from the increased antimicrobial release seen from emulsified ALBC.