

## Small Molecule-Modification of Mixed Calcium Aluminate:Hydroxyapatite Reduces Bacteria Growth

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**Statement of Purpose:** Current biomaterials are frequently unable to meet the vast demands that a physiological environment places on them. For example, a biomaterial should allow for the attachment and growth of specific cell types, depending on its application. In bone scaffold applications the implants aim to enable the attachment and proliferation of osteoblasts, while decreasing or eliminating attachment and proliferation of fibroblasts. Another common issue is the adhesion of bacteria to the implant, formation of biofilms and subsequent infection. If the implant site becomes infected due to biofilm formation, the biomaterial must be removed and replaced requiring additional surgery. These are just two of the desired features of a biomaterial in the physiological environment. For these reasons, many different materials have been modified with growth factors, antibiotics, and other biomolecules to increase material biocompatibility and bioactivity. These molecular modifications add beneficial properties to the biomaterials, but usually only meet one physiological demand that is placed on the material. The goal here is to cast a synthetic calcium aluminate (CaAl) biomaterial, also containing hydroxyapatite (HA). Hydroxyapatite is the same composition as natural human bone, and should increase osteoblast attachment and proliferation. The material can also be subsequently modified, via a linker system, with an immobilized antimicrobial peptide (AMP) or antibiotic that will be useful in resisting bacterial adhesion.

**Methods:** The CaAl:HA composites were cast using three different sizes of calcium aluminum oxide aggregates and hydrated calcium phosphate tribasic. A series of composites containing 1, 2, 3, 4, 5, 10, 15, 20 and 25% HA (by mass) were prepared and cast. After casting, half of the samples were heated at 1000°C for 4 hours. A four-point bending test was used to evaluate the mechanical characteristics of both sets of cast composites. Once the composites have been mechanically characterized they can be chemically modified to immobilize specific biomolecules. 16-heptadecenoic acid was immobilized on the composite using a standard solution deposition surface modification technique. Then, using an alkene-thiol reaction, the antimicrobial peptide of interest can be attached through a sacrificial cysteine residue. Antibiotics, such as vancomycin, are immobilized through amide bond formation with 1,12-dodecanedicarboxylic acid. Once the material has been cast to contain HA and modified with the antibiotic or AMP it can then be evaluated for bacterial adhesion and cell attachment and proliferation.

**Results:** Mechanical evaluation was performed on all composites (%'s HA) using the four-point bending test. Using the four-point test the composites were evaluated for their Young's modulus, flexural strength and failure load. It has been found that at high percentages of HA

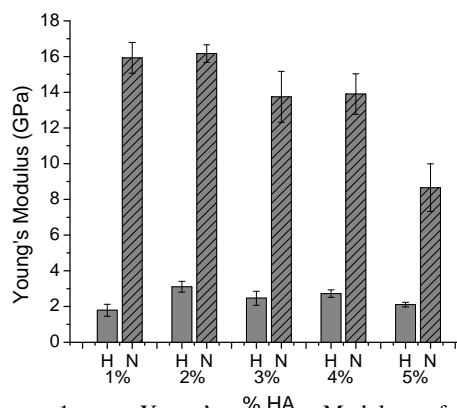


Figure 1. Young's Modulus of composites from 1-5% HA. H=Heated after cast, N=Not heated after cast. At  $p < 0.05$ , all not heated composites are statistically higher.

(>10%) the composites are mechanically weak and break at statistically smaller applied loads (N and Pa). However at smaller percentages of HA (<5%) the composites showed statistically increased mechanical integrity. Additionally it has also been found that the composites that were heated after casting tend to be significantly weaker than those that are not (Figure 1).

Modification with the carboxylic acids on the composite has been successful and is stable through solvent rinse. Additionally, the carboxylic acid molecule is adhered via a mixture of the mono- and bidentate binding modes and the tail group is available at the interface for the alkene-thiol reaction or the amide bond formation. Antibiotics have been successfully immobilized and verified by infrared spectroscopy.

Upon successful immobilization of the AMPs, the composites will be investigated for osteoblast attachment and proliferation. It is hypothesized that the presence of HA within the composite will enhance the osteoblast attachment and proliferation. Additionally the ability of the composite to resist bacterial adhesion will be evaluated using *S. aureus* and *E. Coli*. The antibacterial properties of modified CaAl:HA will be investigated using optical density, microplate fluorescence permeability tests, and scanning electron and confocal laser scanning microscopy.

**Conclusions:** CaAl:HA composites for use in biomaterial applications have been cast and mechanically evaluated for their Young's modulus, flexural strength and failure load. Composites of less than 5% HA were found to be statistically stronger than those of greater than 10% HA. Additionally it has been shown that heating the composites after casting causes a significant decrease in strength. Carboxylic acid molecules have been reacted with the surface to act as linkers for antibiotics or AMPs.