Composite Scaffold for Bone Regeneration with Osteogenic and Antimicrobial Properties
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Abstract
Bioactive scaffolds can be a useful tool for regenerating bone tissue in a critical size bone defect. Scaffolds must have adequate physical and mechanical stability as well as the porosity structure to allow for bone growth on and throughout the scaffold. By creating scaffolds composed of multifunctional materials, additional benefits can be gained such as an increased rate of osteoblast differentiation and bone formation and the prevention of infection. This in vitro study looks to characterize a composite scaffold made of the polymer PLLA, the known osteogenic molecule beta-tricalcium phosphate, and the commonly used antimicrobial agent silver nanoparticles (SNP). The scaffolds were divided into two groups based on density, labeled 10 weight percent (10wt%) and 7wt%. Within each group, the scaffolds were made with varying concentrations of a solution of SNPs ranging from 1ppm as a control to 1500ppm. A variety of tests were completed to analyze the mechanical, structural, and biological properties of the scaffolds based on differences in SNP concentrations. The data shows that increasing concentrations of SNPs produced trends of increased maximum load during mechanical testing, decreased pore size and porosity measured by SEM, and decreased degradation rate and protein deposition after placed in a stromal media for 7 days.

Methods
Scaffolds were created of 7wt% (weight percent) and 10wt% PLLA at 300x and 10wt% PLLA at 33x. Weight percent is defined as the amount of silver present in the scaffold.

Results
The results of the experiments to characterize the composed scaffolds are as follows:
• The mechanical testing (Figure 1) showed that the maximum load was higher for the 10wt% scaffolds and increased with higher concentrations of silver nanoparticles.
• The data from the ICP-OES (Figure 2) confirms that the scaffolds which were consisitent with higher concentrations of silver nanoparticles actually contained more silver than the scaffolds made with the more diluted silver solutions.
• Analysis of SEM photos showed that the 7wt% had a larger pore size which decreased with higher concentrations of silver nanoparticles (Figure 3).
• The porosity, also analyzed by utilizing SEM, also decreased with increasing concentrations of silver nanoparticles (Figure 4).
• From viewing the SEM photos, it can be seen that the scaffold which is made of the polymer PLLA only have a very defined and uniform structure. When viewing the scaffolds which contain beta-tricalcium phosphate alone or with silver nanoparticles, it is clear that the scaffold has a more homogeneous structure.
• The scaffold extraction showed a mass gain after exposure for 1 day (Figure 5) but a mass loss when exposed for 7 days (Figure 6). The trend is that mass loss was decreased from scaffolds with less silver concentration.
• Protein deposition or absorption showed decreased absorption with increased silver concentration when exposed to the stromal media for 7 days (Figure 8).

Conclusions and Future Work
Several reasonable conclusions can be reached from the data which has been gathered throughout this study.
• The 10wt% scaffold is affected less by the addition of the silver nanoparticles. This can be observed by noting the change in pore size, porosity, and mass during degradation studies is less for the 10wt% scaffolds when compared to the 7wt% scaffolds. The smaller effect is most likely due to the increased density of the 10wt% scaffolds. The data from ICP-OES shows that with the addition of the same amount of silver nanoparticles, the percentage of the mass due to silver is less.
• The addition of silver nanoparticles results in the scaffolds having a slower rate of degradation but decreases its ability to absorb protein. The protein deposition is greatly increased in the presence of beta-tricalcium phosphate but with the addition of silver nanoparticles, the phosphate molecules have less of an effect.

Future work involving these composite scaffolds is currently underway. The following characteristics need to be evaluated to fully understand the effects of the addition of silver nanoparticles to a scaffold containing beta-tricalcium phosphate. Expectations are that increased amounts of silver nanoparticles would result in an increased efficacy rate of the killing of microbes but would decrease cell viability and inhibit the osteogenic effects of the beta-tricalcium phosphate.
• The antimicrobial properties are being evaluated by allowing varying scaffolds to be degraded in a stromal media for 7 days then applying bacteria to that media for 1 day. It will then be evaluated for the amount of bacteria present using the Alamar Blue reaction.
• The cell viability/toxicity studies are being conducted using the same technique using human adipose derived stem cells.
• The osteogenic properties need to be evaluated by comparing the expression of specific markers which are increased during osteoblastic bone formation in human adipose derived stem cells when exposed to varying scaffolds.

References