INTRODUCTION:
Bioactive glasses have been studied extensively for use in various clinical applications, including orthopedics. A particular area of investigation is their antibacterial effects on microorganisms. In an aqueous environment, ions including silica, sodium, and calcium are released from the surface of the glass. These reactions facilitate direct bonding to bone by formation of a hydroxyapatite layer in the in vivo environment, allowing the glass to play an active role in bone repair. It has been shown that this layer of soluble ions stimulates osteogenesis by promoting migration, attachment, proliferation, and differentiation of osteoblasts [1]. The same surface reactions facilitating this bioactivity are thought to play a crucial role in the antibacterial activity of bioactive glasses. The aim of this study was to investigate the effects of a proprietary 45S5 bioactive glass when challenged with *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *S. epidermidis*. These bacteria were chosen due to their relevancy for potential infections in a clinical situation. Potential causes for antibacterial effects such as pH and ion release were also investigated.

METHODS:
Four species of bacteria, *E. coli, P. aeruginosa, S. aureus*, and *S. epidermidis*, were suspended in tryptic soy broth (TSB). To simulate bacteriostatic conditions, sterilized glass powder was added at a concentration of 50, 30, or 10 mg bioactive glass/mL broth at the time of inoculation. Control tubes contained no glass particulate. The cultures were shaken at 35ºC and 200 rpm for 24 hours. The bacteria were then plated on tryptic soy agar (TSA) plates and colony forming units were counted after approximately 24 hours. Experiments were performed in triplicate. The bioactive glass utilized is incorporated into a 3-tricalcium phosphate scaffold of Vitoss ® Bioactive Foam (Orthovita, Malvern, PA). The equivalent concentration of bioactive glass contained in the scaffold fell within the 10-50 mg/mL concentration range tested.

pH measurements were taken of 50 mg/mL of bioactive glass in TSB at 37ºC at time intervals of 10 minutes, 1, 6, and 24 hours. Ionic dissolution studies were performed by measuring the ion release from bioactive glass with the scaffold. Samples representing 3.2 mg/mL of bioactive glass were incubated at 37ºC in 50 mL Dulbecco’s Modified Essential Medium (DMEM) for 24 or 72 hours. Dissolved ions were then measured by inductively coupled plasma spectroscopy (ICP).

RESULTS:
Figure 1 demonstrates the viability for the bacterial species at each of the concentrations tested. The *S. epidermidis* was completely killed at all concentrations of bioactive glass. At 30 mg/mL, the viability of the *P. aeruginosa* and *S. aureus* had been reduced to 7 and 24% of the control, respectively. At 50 mg/mL, all of the bacteria were effectively killed.

Table 1 illustrates the rapid rise in pH from approximately neutral to 9.30 within 10 minutes, caused by the addition of glass to the TSB. The pH then increased more slowly to 9.72 after 24 hours. Zero hours represents the pH of the TSB before the addition of glass.

The time dependent release of silica, calcium, and sodium from scaffolds incorporating the bioactive glass is shown in Table 2. The change in sodium over the course of 72 hours is negligible, however both the silica and calcium levels increased over the same time period.

DISCUSSION:
This study has demonstrated that bioactive glass has an appreciable antibacterial viability. This effect may be due to several factors, including the release of ions and rapid shifts in the pH of the surrounding medium [2,3]. Similar to the findings in the current study, Allan et al. found the pH of Bioglass® supernates to rapidly rise to approximately 10 within 1 hour [3]. These authors found a comparable level of antibacterial efficacy between Bioglass supernates and nutrient broth adjusted with NaOH to the same pH, which provides evidence that high pH alone could be responsible for antibacterial effects. The results presented here confirm that a rapid rise in pH is a potential cause for the reduction of bacterial viability observed. Although a high pH environment has been demonstrated to inhibit the growth of bacteria, another study has shown that alkaline conditions have a stimulatory effect on osteoblasts [4], giving further benefit in a scaffold incorporating bioactive glass.

Fluctuations in the ion concentration of the aqueous environment and changes in osmotic pressure may also play a role in the antibacterial mechanism of action of bioactive glass. Ionic dissolution studies presented here demonstrate that the level of soluble calcium and silica ions in the aqueous environment increase within 24 hours. In S53P4 glass, release of ions caused a rise in the salt concentration of the supernate, creating an osmotic pressure that would be inhibitory to most bacteria [3]. Other investigators have speculated that high concentrations of calcium, such as those measured in this study, may cause perturbations in the membrane potential of bacteria, contributing to the antibacterial effects of the glass [5]. Mechanisms of the effects of ion concentrations on bacterial viability should be further investigated.

The results of this study demonstrate that bioactive glass has an appreciable antibacterial effect on a number of clinically important bacteria. In addition to stimulating osteogenesis, the ability of bioactive glasses to inhibit the growth of or kill bacteria commonly found in a clinical situation is an important application of devices which incorporate this material. The proprietary 45S5 bioactive glass used in this study has been added as a component of the clinically proven Vitoss Synthetic Bone Graft scaffold. Numerous clinical benefits may be realized from a scaffold incorporating bioactive glass, including increased rate of bone healing and, as shown here, antibacterial effects in a clinical environment. Future studies will include the investigation of additional antibacterial mechanisms of action of bioactive glass incorporated into a scaffold.

REFERENCES: