INTRODUCTION
Alteration of the geometrical surface configuration of cortical bone may improve incorporation into host bone. However, processing techniques such as demineralization or perforation remove material from the bone and may compromise biomechanical strength. A porous biodegradable scaffold coating that would maintain immediate structural recovery and subsequently allow normal graft healing and remodeling by promoting boning ingrowth could represent an alternative osteoconductive surface modification.

Therefore, we investigated the feasibility of augmenting cortical bone grafts with osteoconductive biodegradable polymeric scaffold coatings. Candidate polymers included poly(propylene fumarate) (PPF), or poly(l-lactic-co-glycolic acid) (PLGA). We report on results of an initial in vivo evaluation of new bone formation around cortical bone samples implanted into the rat tibial implantation model.

MATERIALS AND METHODS
Preparation of Cortical Bone Samples
The experimental bones used in this study were human tibias from young healthy males, obtained from a bone bank. The diaphyses of human tibia bones were cut into uniform slabs of 4-mm length and 1.5-mm thickness. Three types of bone samples were prepared: Type I – cortical bone without coating (control), Type II – cortical bone coated with PLGA-foam, and Type III – cortical bone coated with PPF-foam. Eight samples were included for each of the three types (Types I through III). All grafts were stored until use at -70°C.

Foam Preparation and Coating of Bone Samples
Poly(propylene fumarate) was synthesized by the direct esterification of fumaric acid (Fisher Scientific, Inc.) with propylene glycol (Aldrich Chemical Co., Milwaukee, WI). N-vinylpyrrolidone (VP) (Aldrich Chemical Co., Milwaukee, WI) was vacuum distilled (93 °C, 13 mm Hg) to remove the NaOH inhibitor. All other reagents were obtained from Aldrich Chemical Co., Milwaukee, WI and used as received. PVP(13.24% by wt) was added to a dry powdered mixture of PLGA(52.65% by wt) and TCP(15.24% by wt) to form a viscous putty-like paste. Sodium bicarbonate(2.34% by wt), benzoyl peroxide(2.30% by wt) initiator (Aldrich Chemical Co., Milwaukee, WI), and citric acid (6.87% aqueous, 14.23% by wt) were added resulting in a crosslinked polymer foam coating. During the reaction of citric acid (CA) and sodium bicarbonate (SB) carbon dioxide is formed, which is responsible for foam formation and expansion with respective pore sizes of 100 – 1000 microns. For coating, cortical bone slivers were placed in circular molds and the PPF foaming formulation was added around the bone. Approximately 1.5 grams of the PPF/VP mix was added per bone sample, which then foamed around the bone sample. The vinyl pyrolidone was polymerized and crosslinked the PPF by heating overnight at 70°C. Coated bone samples were then trimmed to a 1-mm thickness of the porous coating.

PLGA-85:15 (lactide:glycolic mole ratio 85:15) was purchased from Boehringer-Ingelheim (Resomer 858) and purified before use by precipitation from an aceton solution (30 mg/ml) into 2-propanol. Glacial acetic acid (gHAc) (Fisher Scientific) was used as received. The PLGA precipitate was created around the bone sample by vacuum evaporation of a solution of purified PLGA in glacial acetic acid (33 mg/ml). This concentration was chosen to achieve a foam density of 40 mg/ml with an average pore size in excess of 100 microns. Thereafter, the solvent was further removed by freeze drying (5 mm Hg, -40°C). This resulted in a porous coating of the bone samples with PLGA (thickness ca. 1 mm).

Animal Studies and Methods of Evaluation:
Three bone graft materials were tested using the rat tibial metaphysis implantation model. NIH guidelines for the care and use of laboratory animals (NIH Publication #85-23 Rev. 1985) have been observed. Adult male Sprague Dawley rats weighing approximately 400 g were used as the animal model. Grafts were implanted into 3 × 5 mm holes that were made into the anteromedial tibial metaphysis of rats. Animals were anesthetized using an intramuscular injection of ketamine HCl (100 mg/kg) and xylazine (5 mg/kg). Three groups of 8 animals each were included in one of two sets of animals. Thus, a total of 48 animals was included in this study. Animals were sacrificed in groups of 8 with groups 1 through 3 of each set being sacrificed at 4 days and at 3 weeks postoperatively.

Following sacrifice, specimens were processed for histologic analysis by fixation in 10% buffered formalin. Specimens were decalcified in EDTA and paraffin embedded. Longitudinal sections (5 μm thick) of the total specimen were then cut and stained with hematoxyline and eosin. Slides were examined for resorptive activity and new bone formation at the implantation site, as well as for inflammatory responses. In addition, histomorphometric evaluation of new bone formation around the different types of grafts was done by acquiring images of serial longitudinal sections of the specimen using a CCD video camera system (TM-745; PULNIX, Synnyvale, CA, U.S.A.) that was mounted on a Zeiss microscope. Images were digitized and analyzed using Image Pro Plus software. For each specimen, the area of newly formed bone surrounding the graft was measured. This measurement was standardized against the total area occupied by the graft in the same section. A minimum of 5 sections obtained from different levels of the specimen was included for this analysis. The spacing between sections of adjacent levels was typically 500 micrometer. This allowed to obtain an approximate absolute volume of the newly formed bone, which is given as an average (mean ± standard deviation) of these volume measures for each bone specimen.

Statistical Analysis
Differences in the amount of new bone formed around the various types of grafts were analyzed for statistical significance by employing an ANOVA test. A p-level of 0.05 was considered statistically significant.

RESULTS
The amount of new bone which formed around the different types of grafts used in this study was significantly higher in the foam-coated groups (Type II and Type III) than in the control group (uncoated; p < 0.02). Although both foam formulations, when coated on cortical bone, were initially equally osteoconductive, PLGA-based foam coatings appeared to have degraded at two weeks postoperatively, whereas PPF-based foam coatings were still present at 4 weeks postoperatively. As a result, the overall amount of newly formed bone around the graft was higher in PPF- than PLGA-coated cortical bone grafts at the end of the 4-week observation period. While significant resorption was present in control allografts with little accompanying reactive new bone formation, PLGA-coated bone grafts showed evidence of bone resorption and subsequent bony ingrowth earlier than those coated with PPF-based foams suggesting that PPF-coated cortical bone grafts were longer protected against host reactions resulting in bone resorption.

DISCUSSION
In this study, we evaluated the feasibility of improving osteoconductive properties of cortical bone grafts by coating them with biodegradable and biocompatible foam scaffolds made from biopolymers. Processing of cortical bone grafts by surface modifications including demineralization and perforation may promote graft incorporation by stimulating bone resorption and subsequent new bone formation in resorptive cavitations. In contrast, surface modification with biodegradable osteoconductive scaffolds could improve graft incorporation by promoting the migration and proliferation of bone forming cells into the graft without appreciable loss of mechanical strength. The biodegradable polymers used in this study seemed to have approximated the three-dimensional nature of bone tissue equivalents. Results of this study further suggested that biopolymeric porous surface coatings can act as an osteoconductive path for bony ingrowth and, hence, should ultimately result in enhancement of incorporation of cortical bone grafts. Both materials had favorable osteoconductive properties as they degrade over time. However, the rate of degradation of the PPF- vs. PLGA-foams was fundamentally different. Thus, the choice of polymer should be based on future clinical repair scenarios, where either rapid or slow resorption of the polymer scaffold is desired. The polymer systems described in the present study offer considerable flexibility for optimizing the osteoconductive properties for different clinical applications.

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