INTRODUCTION:
Osteoconductivity of a graft is defined as its ability to support bone forming cell proliferation and growth throughout the graft. Mineralization and bone tissue formation throughout the graft (i.e., surface & interior) requires fully osteoconductive scaffolds. The lack of such scaffolds has resulted in surface-limited osteoconductivity and osteogenesis, and poses a significant challenge to the scaffold-based bone tissue engineering (BTE) approach. The limited osteoconductivity is mainly due to the constrained scaffold pore size, i.e., ~100 μm and the associated poor transport of oxygen and nutrients to the cells that reside inside the scaffold. PLGA microsphere scaffolds have shown promise as mechanically compatible matrices for bone regeneration. In this study, we addressed the limited osteoconductivity of existing constructs by developing PLGA microsphere scaffolds with increased pore sizes and interconnectivity, referred to as macro-porous scaffolds. Such scaffolds are expected to achieve full osteoconductivity by overcoming the diffusional constraints, and yet, retain mechanical compatibility for effective bone regeneration. The proposed macro-porous scaffolds with enhanced osteoconductivity are highly desirable for effective bone regeneration.

METHODS:
PLGA microsphere scaffolds (10 × 2 mm) with macro-sized pores were fabricated by packing individual microspheres (425–600 μm) and NaCl crystals (200-300 μm) together and heat-sintering at 100°C for 1 hour. All scaffolds were placed in water for 2 hours to leach out the porogen, NaCl. By varying the porogen content, a series of macro-porous scaffolds were fabricated. In this study, we chose PLGA scaffold fabricated with 20wt% porogen as the macro-porous scaffold and 0% porogen as control scaffold. Pore connectivity and accessible pore volume were measured using micro-CT imaging and analysis (Scanco μCT40). For in vitro evaluation, we seeded MC3T3-E1 mouse pre-osteoblastic cells on the scaffolds, and cultured in mineralization media (MEM-α media supplemented with 10% FBS, 50 μg/mL ascorbic acid, 3 mM β-glycerophosphate, and 1% penicillin/streptomycin). The culture was maintained for 7, 14, 21, and 28 days in an incubator at 37°C, 5% CO₂ and 95% humidified air. Cell proliferation on control and macro-porous PLGA scaffolds (n=3/group) were evaluated quantitatively using PicoGreen DNA assay. We examined matrix mineralization via Alizarin Red staining and histology analysis. Hematoxylin staining and protein expression of bone markers (i.e., osteopontin). Statistical analysis was performed using a one-way ANOVA.

RESULTS:
Macro-porous PLGA scaffolds display significantly higher pore sizes (i.e., 200-800 μm) and interconnectivity than control scaffolds (Fig. 1), two aspects required for enhanced osteogenesis and angiogenesis in bone tissue engineering. 2D MicroCT images (Fig. 1A) demonstrate the increasing porosity and mean pore size in macro-porous scaffolds. Figure 1B illustrates all scaffold pores accessible from the outside at a cut-off dimension of 200 μm for control and macro-porous scaffolds (i.e., top and bottom images, respectively), whereby grey area represents accessible pore space. Increasing porosity results in increased interconnectivity, and the accessible volume available for cell infiltration (Fig. 1C). For instance, a sphere with a diameter of 200 μm can access 20% of the total pore volume within the control PLGA scaffold, whereas the same sphere can access >80% of the pore volume of the macro-porous scaffold. Furthermore, the compressive modulus and strength of macro-porous scaffolds, 248.6 ± 63.3 MPa and 4.8 ± 1.8 MPa respectively, although lower than the control scaffold, are in the range of human cancellous bone mechanical properties.

We also confirmed the ability of macro-porous PLGA scaffolds to promote cell infiltration. After 28 days of culturing MC3T3 cells on the scaffolds, we performed histology and IHC (Fig. 2). Hematoxylin staining highlighted cells densely located on the top of control scaffolds and not in the center of the scaffolds (Fig. 2A). On the other hand, macro-porous scaffolds displayed cell proliferation on the surface, as well as increased cell infiltration and proliferation in the center of the scaffold (Fig. 2B, C). Likewise, we found osteopontin expression only on the surface of the control scaffolds (Fig 2D). However, macro-porous PLGA scaffolds exhibited cells expressing osteopontin at the top, as well as the center of the scaffold (Fig. 2E, F).

Macro-porous scaffolds showed significantly enhanced mineralization potential compared to control scaffolds (Fig. 3). After 14 and 28 days of culturing pre-osteoblastic MC3T3 cells on control and macro-porous PLGA scaffolds, we performed Alizarin Red staining. The control scaffolds displayed mineralization limited to only the scaffold surface. On the other hand, scaffolds with macro-sized pores showed mineralization occurring throughout the scaffold (i.e. top, center and bottom). Furthermore, Alizarin Red quantification demonstrated significantly more mineralization in macro-porous scaffolds than control after 14 and 28 days, 48.2% and 38.8% respectively.

DISCUSSION:
We have developed a new set of biodegradable scaffolds with increased porosity, and human cancellous bone -mechanical compatibility for bone defect repair/regeneration. The macro-porous scaffolds have demonstrated their fully osteoconductive nature by displaying osteoblast survival and mineralization throughout the scaffold (i.e. surface and interior). Provided the osteoinductive environment, macro-porous scaffolds may promote homogeneous and enhanced bone regeneration throughout the scaffold. Unlike bioreactor culture methods, which are complex in nature and only effective in vitro, macro-porous scaffold development is simple and their use can be effective both in vitro and in vivo. In addition, the increased pore size is expected to improve osteoclast participation, and hence, bone remodeling to regenerate bone more closely to the natural repair process in vivo. Therefore, the newly designed macro-porous scaffolds with enhanced osteoconductivity would address a significant challenge in the field of scaffold-based bone tissue engineering and its clinical applicability towards bone defect repair and regeneration.

REFERENCES:

Figure 1: (A) 2D micro-CT images of control (top) and macro-porous (bottom) PLGA scaffolds. (B) Porosity analysis illustrating pore interconnectivity (grey color) with only the pores ≥200 μm, and (C) measuring the accessible volume of the scaffolds.

Figure 2: (A-C) Hematoxylin stain, & (D-F) OPN immunostain of MC3T3-E1 cells seeded on control & macro-porous scaffolds; arrow highlighting cells & OPN expression.

Figure 3: Mineralization potential of control and macro-porous scaffolds.