Development and Evaluation of Optimized Scaffolds Pre-seeded with Effective Progenitor Combination for Vascularized Bone Regeneration

INTRODUCTION:
Treatment of critically sized bone defects presents a significant challenge in orthopaedic surgery. Tissue engineering approaches are highly promising, but limited vascularization has largely restricted its current success, as rapid vascularization upon implantation is a prerequisite to bone regeneration throughout the construct. Here, we investigated a novel combination of optimally-designed biodegradable scaffolds seeded with an effective combination of progenitor cells. Here, we illustrate significantly improved mesenchymal stem cell (MSC) survival on our optimized scaffolds in long-term in vitro culture. Further, we demonstrate a synergistic increase of BMP-2 and VEGF expression, key osteogenic and vasculogenic markers, in MSCs and endothelial progenitor cells (EPCs) seeded on our optimized scaffolds in vitro, and enhanced bone and vascular formation implanted subcutaneously 6 weeks in vivo.

METHODS:
Poly(85 lactide-co-15 glycolide), PLGA macro-porous scaffolds were fabricated by packing individual microspheres (425–600 μm) and NaCl (200-300 μm) at a weight ratio of 80:20, heat sintering at 100°C for 1 hour, and soaking in water for 2 hours. Control PLGA scaffolds were similarly fabricated, except NaCl was not be added prior to sintering. Pore connectivity and accessible pore volume were measured via MicroCT.

In vitro cell viability was evaluated by culturing rabbit MSCs on macro-porous and control scaffolds (5 mm diameter x 10 mm height) in osteogenic media (DMEM, 10% FBS, 1% ITS, 10 nM DEX, 10 mM f-glycerophosphate, 10 μg/ml ascorbic acid). The culture was maintained for 7 and 21 days, at which point scaffolds were bisected lengthwise and cell viability (Live/Dead Assay) analysis was performed, and imaged via confocal microscopy.

Rabbit MSCs and peripheral blood-derived EPCs were isolated. 250,000 MSCs, EPCs and co-cultured MSCs and EPCs were cultured on each scaffold in a mixed media (i.e., one part endothelial cell growth media-2 and one part osteogenic media). After 48 hours, we performed RT-PCR for BMP2 and VEGFA expression analysis. At this 48 hour time-point, we also implanted cell-seeded control and macro-porous constructs subcutaneously in SCID mice for 6 weeks, at which point Masson’s Tri-chrome staining was performed to assess osteogenesis and angiogenesis levels.

RESULTS:
Figure 1. (A) Scaffold Accessible Volume: MicroCT analysis demonstrating increased accessible volume of macro-porous scaffolds compared to control scaffolds. (B) In Vitro Cell Viability: Live/Dead assay demonstrating robust survival of MSCs in the interior region of the construct after 21 days in vitro, compared to control scaffolds that displayed nearly all dead/apoptotic cells. Green and red represent live and dead cells, respectively. Scale bar = 200 μm.

MicroCT analysis data illustrating scaffold pore interconnectivity are presented as a function of pore size, providing direct measurements of externally accessible pore space through the full range of diametral pore dimension (Fig. 1A). For example, over 30% of macro-porous scaffold is associated with pores sizes >300 μm, whereas only 12% of control scaffold is accessible at the pore size dimension. Macro-porous scaffolds displayed increased accessible interconnected volume allowing for enhanced visualization and cell infiltration throughout the scaffold, which still retaining significant mechanical strength in the range of human cancellous bone (data not shown). In addition, after 21 days in vitro, we observed significant MSC-derived osteoprogenitor cell proliferation and survival in the interior region of the construct, as compared to control scaffolds (Fig. 1B).

DISCUSSION:
Our dual approach involves the design and identification of optimal scaffolds and effective progenitor cells for effective bone regeneration. The optimally-designed (i.e., macro-porous and biomechanically compatible) biodegradable scaffolds demonstrate bone and blood vessel forming cell survival and growth throughout the matrix pore size.

In addition, the osteo- and vascular progenitor combination will enhance the vasculogenic and osteogenic potential by synergistically working together and producing elevated levels of BMP-2 and VEGF. This dual approach combining the optimal matrix, and osteo- and endothelial-progenitor cells will offer an effective strategy for vascularized bone formation in a large or critical sized bone defect. Here, we have demonstrated compelling evidence of the feasibility of designing optimal matrices, and the development of effective progenitors. Our approach will address a significant challenge in the field of scaffold-based bone tissue engineering and its clinical applicability towards critically-sized bone defect regeneration.

SIGNIFICANCE:
Presently, there are no practical and effective solutions for clinical critically-sized area bone defect regeneration. Our novel approach, which involves seeding the appropriate vascular and osteo-progenitor cells (i.e., PB-EPCs, MSCs) on optimally-porous, biomechanically strong scaffolds, demonstrates significantly enhanced vascularization and is expected to offer an efficient treatment option for critically-sized bone defect regeneration.

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REFERENCES: