DESIGN OF A 3D PERFUSED CELL CULTURE SYSTEM TO EVALUATE BONE REGENERATION TECHNOLOGIES

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Introduction
The purpose of this project is to develop a three-dimensional in vitro bone bioreactor system to investigate cell seeding of 3D matrices, bone cell-ECM interactions, and the effects of mechanical and biochemical influences on ECM synthesis, organization, and mineralization. Culture conditions currently employed only allow a small fraction of the available cells to differentiate and secrete an organized extracellular matrix resembling bone. In addition, non-physiologic concentrations of β-glycerophosphate have been required as a media supplement to achieve reproducible mineralization of the extracellular matrix. A few recent studies have suggested that a three dimensional substrate may be required to enhance cellular responses and matrix formation1. Our culture system provides the opportunity to investigate cell function and matrix synthesis within 3D substrates under controlled in vitro loading conditions (Fig. 1 and 2). In addition, a 3D culture system within a regulated mechanical environment will be useful for examining the interactions between bone-forming cells and the extracellular matrix they produce as well as the differential effects of various cell types/sources and soluble or matrix-bound factors on bone formation. Finally, this in vitro model system will serve to screen cell and scaffold technologies prior to integration into animal model systems. This apparatus has the capacity to perfuse media through cell seeded cylindrical constructs, measuring 0.25 x 0.25 inches, at rates between 0 and 100 ml/min. Additionally, we are able to apply loads up to 25lbs. to each construct at a frequency between 0 and 30 Hz. Load and displacement are measured and controlled by a load cell and LVDT feedback loop. In this study we have performed preliminary perfusion experiments only (no loading) in order to optimize the system and the perfusion rates used.

Methods
Thirty two human freeze-dried trabecular bone cylinders measuring 0.25” x 0.25” were seeded with MC3T3-E1 osteoblast-like cells each. Two million cells were suspended in 100μl of culture media, placed directly onto the trabecular samples and incubated at 37°C for 15 minutes. After this time, 3ml of media was added to the samples, covering them entirely. Twenty four were placed in the flow chambers supplied with 15ml of media and the other 8 were grown in static culture with 10ml media. The media was α-MEM with 10% FBS and 1% Pen. Strep. supplemented with 3mM β-glycerophosphate, 10-8 M dexamethasone, and 50 μg/ml ascorbate. Media was changed at 2 day and 4 day intervals in both the static culture and the perfusion system. Eight cell seeded constructs were perfused at a rate of 0.1ml/min, another eight were perfused at 0.2ml/min. and another eight at 1ml/min for one week. The constructs were also viewed with a laser scanning confocal microscope (LSCM) for cell viability. Haemotoxylin and eosin stains of histologically prepared sections of each perfusion rate were also prepared. Cell viability of a 1 day cell seeded construct prior to experimental analysis was also performed. Osteonectin (ONN), osteopontin (OPN), osteocalcin (OCN), alkaline phosphatase (ALP), bone sialoprotein (BSP) and house keeping gene GAPDH presence was analyzed by RT-PCR at the one week time point.

Results
Figure 3a illustrates the structure of the porous trabecular bone with attached cells 1 day after seeding. One week perfusion of cell seeded trabecular bone at 0.1ml/min. allowed viable cells to proliferate (Fig. 3b) whereas a rate of 0.2ml/min. produced a greater proportion of non-viable cells. This perfusion rate of 1ml/min resulted in complete cell necrosis. In all cases, cells were seeded throughout the porous matrix. Histology confirmed the cell presence at the center of the trabecular bone as well as at the periphery. RT-PCR showed production of GADPH, ONN, OPN, OCN, ALP and BSP at one week in both the static culture and the 0.1ml/min perfused culture. The remaining viable cells from the 0.2ml/min perfusion also produced the above phenotype (Fig. 4).

Discussion
A 3D perfusion cell culture system has been developed to evaluate skeletal regeneration technologies within a controlled in vitro mechanical environment. It is apparent that perfusion rates above 0.2ml/min are detrimental to cell viability on trabecular bone graft scaffolds in this novel 3D system. Although osteoblastic phenotypes were demonstrated by the cells with 0.2ml/min perfusion rates with RT-PCR, the LSCM images verified a large proportion of cell necrosis, possibly due to cell shearing from the higher perfusion rate. This perfusion system may provide an effective way of providing fresh media to cell seeded 3D porous constructs. The perfusion rate must be kept to a minimum in this system in order to prevent cell mortality. Experiments to evaluate the combined effects of perfusion and cyclic mechanical loading on cell-seeded scaffolds are currently underway.

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Reference