INTRODUCTION
The role of the biomechanical environment in bone growth and remodeling has been well recognized. Although the mechanisms involved in mechanotransduction may be multi-factorial, it is well accepted that mechanosensitive ion channels play an early role in responding to the strain and fluid flow environment (El Haj et al. 1999). These channels respond to flow and strain profiles by transmitting signals across the membrane to activate downstream signaling and gene expression resulting in matrix production and remodeling. Mechanical conditioning has been proposed for preconditioning of stem cell seeded constructs and ultimately producing tissue engineered bone. In this study, we have set out to not only augment the load signal, but to use our bioactive materials which we term ‘mechano-active’. The aim of this project is to create a biodegradable material containing slow release agonists which enhance the load signal and stimulate increased matrix synthesis. The advantage of this system is two-fold: the matrix is laid down in the region according to the mechanical signals, and the control of the augmentation is limited to a mechanical switch rather than continuous as is the case of bioactive growth factor-containing scaffolds. The calcium channel agonist used in this project is Bay K8644 (Bay), an L-type Ca$^{2+}$ channel agonist which prolongs single channel opening time once it is activated via external cues.

METHODS
Using human trabecular derived bone cells from tibial fracture or distraction osteogenesis (10-30 yr) patients or bone marrow stromal cells derived from rats, we report on the results of our investigations into the effects of the Bay release scaffolds in vitro in 3D culture systems using a perfusion/compression bioreactor and in vivo in a rat model subjected to 4 point bending. Human trabecular bone derived cells were cultured with media containing osteogenic supplements of dexamethasone (10^{-8} M), ascorbic acid (50 mg/ml) and beta-glycerophosphate (10 mM) and seeded in 3D porous PLLA scaffolds with and without the Bay. The porous three-dimensional PLLA scaffolds were fabricated using a standard salt leaching technique with sodium chloride as the leachable component to create 4 mm height x 10 mm diameter cylinders with a pore size of 250-350 microns. The cell-scaffold constructs were cultured with media containing the osteogenic supplements for 3-4 weeks then perfused or perfused/compressed for one week at 0.1ml/min perfusion rate. The cyclic load strain was applied 1 hour per day at approximately 0.5% strain level and 1 Hz frequency. Real-time PCR was performed to determine the expression of mRNA produced of bone-related gene collagen type I and core binding factor a1 (CBFA-1). The specimens were also embedded in resin and the sections were stained with Von Kossa for detection of mineralised bone matrix. In vivo work was carried out in a rat model. Bone marrow stem cells were harvested from long bones of donor rats. Following expansion to passage 2, the cells were seeded to above described PLLA scaffold (p2 x 5 mm) and cultured for 4 days. A 2x5 mm defect was made at the anterior lateral side of the right tibia in rats. The cell-scaffold constructs were inserted to the defects. At day 8 after surgery, the operated leg (tibia) was stimulated mechanically by a four-point bending machine (Robling, 2001). A sinusoidal load was applied for 300 cycles at 2 Hz with a maximum load of 40 N. The rat received tetracylin labeling at day 13 and 21, and were sacrificed at day 23 following surgery. The implants were evaluated using histomorphometry and micro-CT.

RESULTS
Our results demonstrate the enhancing effects of biomechanical conditioning on bone matrix production in a perfusion/compression bioreactor. In the presence of chemical agonists which augment the load signal, we can further enhance the production of bone. Figure 1a shows the human bone cells grown within the PLLA porous matrix following static conditioning for 4 weeks. The agonist was released from the scaffolds in a controlled manner in vitro. Figure 2 shows that after 40 days‘ incubation, the released agonist concentration was still within physiological active level. Von Kossa staining of the constructs is presented in Figure 3 showing the calcium deposition. RT-PCR results, Figure 4, demonstrated that the sample group of cells conditions showed a trend towards up-regulating gene expression in the experimental group in comparison to the controls. In addition, we will report on implantation of our constructs in in vivo studies using 4 point bending rat model. Furthermore, following 4 point bending of implanted stem cell-seeded, Bay-containing constructs in in vivo rat models resulted in PLLA scaffolds being rapidly invaded by soft tissue with new bone formation occurring preferentially at the endosteal interface of the scaffolds and intact bone.

DISCUSSION
Our results show that addition of the agonist for slow release is sufficient to enhance the load-related responses in bone cells within the scaffolds, specifically collagen type I expression. In addition, we have demonstrated that the scaffolds with agonist can be implanted in vivo in a rat model. Extracellular matrix was produced within the scaffolds and seeded cells remained for 2 weeks encouraging further tissue production. As the agonists only act when the calcium channels are open by attenuating the calcium flux, the stimulation is specifically targeted to scaffolds subjected to load either in vitro or ultimately in vivo. Our results suggest that manipulating mechanosensitive ion channels and attenuating the opening of calcium channels may be an effective strategy for amplifying matrix production via mechanical stimulation. This may be applied to bone tissue engineering and potentially engineering of other load-bearing connective tissues.

REFERENCES

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