

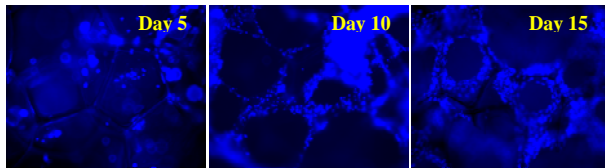
A 3-dimensional, in vitro model to study the effects of compressive loading on osteoblastic cells.

Anuphan Sittichokechaiwut¹, Anthony J. Ryan² and Gwendolen Reilly¹

¹ Department of Engineering Materials, ² Department of Chemistry University of Sheffield, UK

INTRODUCTION: Mechanical force is an osteoinductive factor that plays an important role in bone growth and repair in vivo¹. Our aim was to design a model system by which bone cell responses to mechanical load could be examined in vitro in a 3D environment. Our previous study has shown that osteoblastic cells survive well and proliferate in our polyurethane (PU) open cell foam scaffolds. Cell number after 15 days of culture was four times that after 5 days of culture. Examination of cell distribution, under fluorescence microscopy, showed that cells were clearly adherent and spread out along the sides of the pores of the PU and were seen in the centre of a 25 mm diameter, 10mm high scaffold (Fig.1).

Fig 1: Cell distribution on PU scaffold by using Fluorescence microscopy (DAPI staining).



METHODS: MLO-A5 osteoblastic cells were seeded at densities of 250,000 cells per scaffold in cylindrical polyurethane (PU) scaffolds, 10 mm thick and 10 mm diameter. The cell seeded PU scaffolds were dynamically loaded in compression at 1Hz, 5% strain in a sterile fluid-filled chamber (Fig.2). Loading was applied for 2 hours per day at days 5, 7 and 9 of culture. Between loading cycles, scaffolds were cultured in an incubator in standard conditions. Cell seeded scaffolds were assayed at day 3 and day 11 for cell proliferation by MTS assay

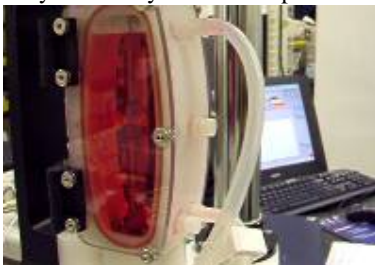


Fig. 2 Bose ElectroForce 3200 with biodynamic chamber

of cell viability and collagen by Sirius red.

RESULTS: Osteoblastic cells survived in loaded scaffold, final cell number was slightly but not significantly lower in loaded samples compared with unloaded at day 11 (Fig 3). In contrast, collagen content increased in loaded scaffolds. Microscopy of Sirius red stained scaffolds showed much more staining in the loaded group on day 11 (Fig 4). Sirius red quantification indicated that in loaded samples collagen content increased by 66% between days 3 and 11, compared

with only 44% in unloaded controls. The scaffold stiffness (Young's modulus) also increased in loaded samples over time (Fig 5).

DISCUSSION & CONCLUSIONS: The goal of this

Fig 3: Cell viability over 11 days (MTS assay)

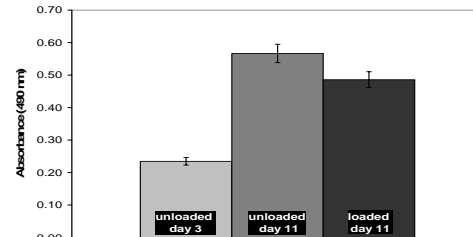


Fig. 4: Collagen on PU scaffold by using light microscopy (Sirius red staining) on day 11.

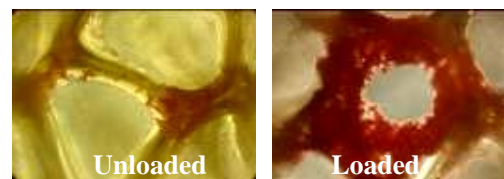
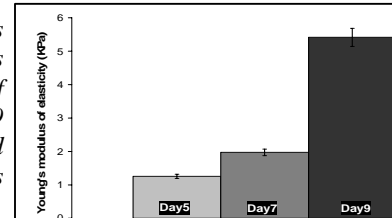


Fig 5: Stiffness (Young's modulus) of Scaffolds over 9 day on loaded specimens



study was to develop a model to analyse the effects of mechanical stimulation on osteoblastic cells in a 3-D system, to understand how mechanical stimulation can enhance bone tissue engineering. Although the number of viable cells decreased under our loading regimen, the amount of collagen and scaffold stiffness increased, indicating increased matrix production by cells. This model has the potential to answer questions about cell survival, distribution, matrix production and stiffness in 3-D, in response to mechanical signals.

REFERENCES: ¹ Carter, D.R et al (1988), J. Orthop. Res. 6(5):736-748. ² Y. Kato et al (2001), J Bone Miner Res. 16(9):1622-33.

ACKNOWLEDGEMENTS: Dr. L. Bonewald, University of Missouri at Kansas City, UA, kindly donated MLO-A5 osteoblastic cell. Bose provided use of the ElectroForce 3200 for an evaluation period. Funding for this project was provided by the Royal Society of London, The University of Sheffield and the Thai Government.