

## Use of Simvastatin for Enhanced 2D and 3D Bone Tissue Engineering Constructs

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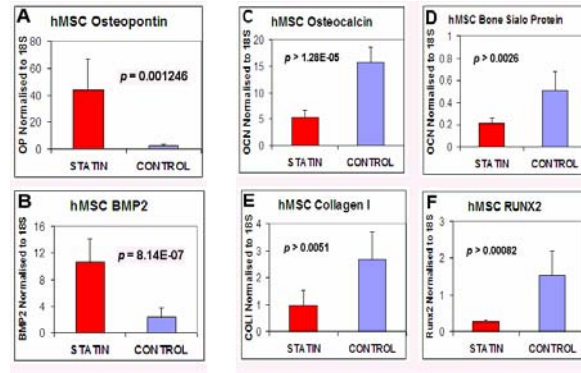
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**INTRODUCTION:** Bone tissue engineering is a potential emerging therapy for patients suffering from bone loss as a result of trauma or disease. Statins are a commonly prescribed cholesterol lowering drug, however it has recently been shown that they also have the beneficial side effect of enhancing bone matrix formation(1). The inhibition of the cholesterol biosynthesis pathway by statins also interacts with other pathways to produce an upregulation of BMP2. This study has looked at using this bone enhancing effect of statins for the novel use in tissue engineered scaffold constructs. A 5 $\mu$ M concentration of Simvastatin was added as a supplement to media of human mesenchymal stem cells (hMSC's and human osteoblasts (hOB's) which had been seeded in the 2D cultured onto 6 well plates and cultured for 7days *in vitro* and the gene expression of various bone related genes analysed. In the 3D culture the cells were seeded onto PLLA scaffolds and cultured *in vitro* with Simvastatin over a 7-week period, then the volume and location of mineralized matrix were analysed.

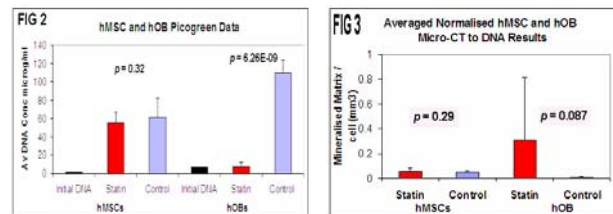
**METHODS:** 2D culture: MSCs and hOBs seeded at 100,000 cells/well. Media: DMEM for hMSCs,  $\alpha$ MEM for hOBs. 5 $\mu$ M simvastatin supplement to media, controls no simvastatin. Media supplements: 0.1mM human sera, 0.01mM antibiotic, 0.01mM ascorbic acid, 0.01mM  $\beta$ -Glycerophosphate. Real-time-RTPCR was used to analyse expression of Osteopontin (OP), Bone-morphogenic-protein-2 (BMP2), Osteocalcin (OCN), RUNX2, Collagen II (Coll II) and bone-sialoprotein (BSP) normalised to 18S. 3D culture: PLLA scaffolds (porosity of 88 $\pm$ 2%, pore size of 250-350 $\mu$ m, manufactured using a salt leaching technique). hMSC's seeded at ~200,000 cells/scaffold, hOB's at ~850,000 cells/scaffold. Media as above, again with 5 $\mu$ M simvastatin added as a supplement to media. Scanco microCT40 was used to determine the volume and location of matrix mineralization. A picogreen DNA assay was used to determine DNA concentration.

**RESULTS:** 2D culture: hMSC and hOB gene expression of OP and BMP2 significantly increased in the statin group, whilst OCN, BSP, Coll II and RUNX2 showed significant or greatly suppressed expression. 3D culture: hMSC's, no significant difference between statin and control groups for volume of mineralised matrix (VMM) and rate of proliferation. Normalisation of VMM to DNA also showed no significant difference. hOB's, no significant difference between statin and control VMM, however the rate of proliferation was significantly lower in the statin group ( $p=6.26 \times 10^{-9}$ ).

Normalisation of the VMM to DNA revealed a trend of increased VMM being produced per cell in the statin group ( $p=0.087$ ).



**FIGURE 1A-F** 7day real time bone related gene expression of human MSC's with or without exposure to simvastatin



**FIGURE 2** hMSC and hOB DNA results 7week culture on 3D PLLA scaffolds with and without exposure to simvastatin

**FIGURE 3** hMSC and hOB microCT mineralised matrix results normalised to DNA, 7week culture on 3D PLLA scaffolds with and without exposure to simvastatin

**DISCUSSION:** Results suggest that in the long-term 3D cultures, 5 $\mu$ M simvastatin produces a trend of increased VMM/cell in hOBs whilst causing a significant reduction in cell proliferation, whilst this was not seen in the 3D hMSC cultures. Simvastatin when added to hMSC and hOB 2D cultures significantly upregulated expression in genes that are used as markers of osteoblastic differentiation and mineralization, whilst down regulating others. This suggests that statins play a role in altering the coupled regulation, of cell cycle progression and osteogenic differential gene expression. Further studies are underway to optimise the type, concentration and timing interval of statin addition to culture, in human cells so that more significant effects can be produced and the mechanisms of action better understood. We anticipate that optimising the statin administration timing to 2D/3D bone cell constructs to allow for cell proliferation prior to statin induced osteoblast maturation has great potential for bone tissue engineering applications.

**REFERENCES:** (1) Mundy, Garrett, Harris, Chan, Chen, Rossini, Boyce, Zhao, Gutierrez (1999) Science 286: 1946-1949.