# Chondrogenesis of Human Bone Marrow Mesenchymal Stem Cells is Modulated by Frequency and Amplitude of Dynamic Compression and Shear Stress

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**INTRODUCTION:** A number of studies have shown a beneficial effect of load for chondrogenesis of MSCs. Computational models predict that the final outcome of MSC fate is determined by a combination of tissue shear strain and fluid flow [1-2]. One potential reason that *in vitro* MSC chondrogenesis commonly leads to terminal hypertrophy is the fact that the developing tissue is not stimulated. Under these conditions the models predict that bone will be formed. Addition of low amounts of tissue shear strain and fluid flow is predicted to result in cartilage, whereas higher tissue shear strain and fluid flow is predicted to result in fibrous connective tissue. However, the models were mainly established for fracture healing. The present study aimed to test if this theory is applicable in the differentiation of *in vitro* MSC-laden scaffolds, and to identify the optimal load regimen which enhances the differentiation of cells into a chondrocytic phenotype.

**METHODS:** P3 human bone marrow MSCs were seeded into fibrin-polyurethane scaffolds (8mm×4mm) at a density of $5\times10^6$ per scaffold. Cell-scaffold constructs were cultured in ITS+DMEM containing 1 ng/ml TGF-β1. They were pre-cultured for 7 days, then loaded 1 hour daily for 7 days. Samples were divided into 7 groups: 1 control group and 6 loaded groups with different load regimens (table 1).

Measurements included DNA, glycosaminoglycan and mRNA expression of collagen type I, II, and X, aggrecan, TGFB1, TGFB3, and Sp7.

**RESULTS:** Under the same frequency, the peak load force doubled accordingly when the amplitude doubled from 5% to 10% and from 10% to 20%. Under the same amplitude, the 1 Hz frequency induced 2-20% higher peak load force compared with the frequency of 0.1 Hz. Higher load frequency and higher compression amplitude induced higher GAG synthesis,

*Table 1. Description and abbreviation of 6 loaded groups.*

| Ball Rotation of ±25° and Axial Compression (superimposed on 10% static offset strain) | | |
|---|---|---|
| Abbreviation | Rotation and Compression Frequency (Hz) | Compression Amplitude (strain) |
| 0.1Hz 5% | 0.1 | 5% |
| 0.1Hz 10% | 0.1 | 10% |
| 0.1Hz 20% | 0.1 | 20% |
| 1Hz 5% | 1 | 5% |
| 1Hz 10% | 1 | 10% |
| 1Hz 20% | 1 | 20% |

higher chondrogenic gene expression, higher COL2/COL1, AGG/COL1, COL2/COL10, AGG/COL10, and AGG/Sp7 gene expression ratios, as well as higher TGFB1 and TGFB3 gene expression.

**DISCUSSION & CONCLUSIONS:** Application of dynamic compression and surface shear enhanced chondrogenesis of hMSCs compared with control samples. Culture of 14 days *in vitro* can not change the Young's modulus or the permeability of scaffolds as much as that predicted in the fracture healing model. To improve understanding a long-term study with samples at different time points should be carried out. Within the selected range of load, higher frequency and higher amplitude are superior for chondrogenesis. Load regimens of 0.1Hz 20%, 1Hz 10% and 1Hz 20% had the best and comparable beneficial effect on chondrogenesis of hMSCs.

**REFERENCES:** [1] Prendergast et al., J Biomech. 1997;30:539-48. [2] Kelly et al., J Biomech. 2005;38:1413-22.

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