

Mechanical Load Promotes Chondrogenesis of Human Mesenchymal Stem Cells through the TGF- β Pathway

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INTRODUCTION: Mesenchymal stem cells derived from bone marrow (MSCs) have the potential to differentiate into chondrocytes. This study investigated the effect of dynamic compression and surface rotation on hMSC chondrogenesis. We also investigated the influence of transforming growth factor β 1 (TGF- β 1) on the response of hMSCs to load.

METHODS: P3 human bone marrow MSCs were seeded into fibrin-polyurethane scaffolds (8 mm \times 4 mm) at a density of 5×10^6 per scaffold. Cell-scaffold constructs were cultured in ITS+DMEM containing either 0, 1, or 10 ng/ml TGF- β 1. They were pre-cultured for 7 days, then loaded 1 hour daily over 7 consecutive days. Cyclic axial compression (10-20% of scaffold height, sinusoidal strain, 1Hz) was performed by a ceramic hip ball 32 mm in diameter. Oscillation of the ball over the construct surface was applied ($\pm 25^\circ$, 1 Hz). Unloaded constructs served as controls.

A series of experiments were done in combination of mechanical load and TGF- β receptor-I selective inhibitor LY364947 to investigate the role of TGF- β signaling.

Measurements included DNA, glycosaminoglycan and mRNA expression of collagen type I, II, and X, aggrecan, TGF β 1, TGF β 3, proteoglycan-4, and Sp7. TGF- β 1 and TGF- β 3 protein were measured by ELISA.

RESULTS: Load stimulated GAG synthesis and significantly increased the expression of all chondrogenic markers in the absence of added TGF- β 1. The effect on gene expression was far smaller when 10ng/ml TGF- β 1 was added and intermediate with 1 ng/ml. Without added TGF- β 1, load significantly up-regulated TGF- β 1 and TGF- β 3 protein synthesis. While with 10ng/ml TGF- β 1 in the medium, load decreased TGF- β 1 protein synthesis by 20%. Addition of LY364947 significantly reduced the chondrogenic gene expression compared with vehicle control.

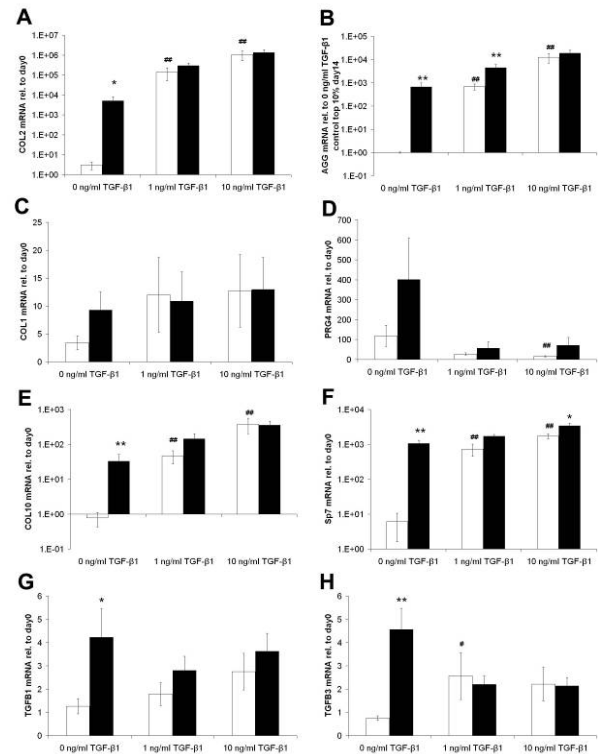


Fig. 1: Relative mRNA expression of hMSCs cultured in the top 10% sections of scaffolds for 14 days with (load-black) or without (control-white) load on days 8-14. Mean \pm S.E.M., $n=6$. # $P < 0.05$, ## $P < 0.01$ vs control (0 ng/ml TGF- β 1). * $P < 0.05$, ** $P < 0.01$ vs respective control samples for a given medium condition.

DISCUSSION & CONCLUSIONS: Different TGF- β 1 concentrations influenced the effect of mechanical load on the chondrogenesis of hMSCs, with the greatest effects being seen at lower TGF- β 1 concentrations. Under lower concentrations of TGF- β 1, load up-regulated TGF- β gene expression and TGF- β 1 protein synthesis. Blocking of TGF- β receptor-I signaling abrogated this response. This indicates the mechanically induced chondrogenesis of hMSCs is an outcome of increased TGF- β synthesis and signaling.

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