Mimicking the Stem Cell Microenvironment: The role of mechanical load on the chondrogenesis of human bone derived mesenchymal stem cells in fibrin-polyurethane scaffolds

Stoddart MJ., Li Z., Eglin D., Alini M.
AO Research Institute, Clavadelerstrasse 8, Davos Platz, Switzerland

Statement of purpose: The differentiation of stem cells into chondrocytes is highly dependant on the signals the cells receive. TGFβ is used to induce chondrogenesis and yet this would not be supplemented within an articular defect. We have developed a biodegradable polyurethane-fibrin scaffold system which has been shown to be highly favorable for chondrogenesis under classical chondrogenic stimuli (i.e. TGFβ containing medium). The aim of this study was to determine the effectiveness of this scaffold composite in supporting chondrogenesis in the absence of an exogenous TGFβ signal, but under the influence of a loading regime similar to that which might be experienced during a patient rehabilitation protocol. To achieve this we seeded human mesenchymal stem cells (hMSCs) into the polyurethane-fibrin scaffold and applied compression and sheer using a novel bioreactor. Groups containing TGFβ were used as a control.

Methods: The scaffolds were prepared by a salt leaching-phase inverse technique consisting of the mixing in equal weight of a porogen (sodium phosphate heptahydrate dibasic salt, particles size range from 90 to 300 μm) with a solution containing a mixture of solvents and the polyurethane synthesized from hexamethylene diisocyanate, poly(epsilon-caprolactone) diol and 1,4:3,6-dianhydro-D-sorbitol in a one step solution polycondensation reaction. ***Culture of hMSCs*** P3 hMSCs were suspended in fibrin and seeded at a cell density of $5x10^6$ per polyurethane scaffold (8 mm × 4 mm). All groups were cultured in medium consisting of DMEM, ITS, Pen/Strep, ascorbate-2-phosphate, 5 μM ε-amino-caproic acid, and 10^{-7} M dexamethasone. 0 ng/ml, 1 ng/ml, or 10 ng/ml recombinant human TGF-β1 was added into the medium of 3 groups respectively prior to and during mechanical loading. ***Mechanical Load.*** Mechanical conditioning of cell-scaffold constructs was performed using our previously described bioreactor system[5]. The loaded group was exposed to ball oscillation of ±25° at 1 Hz and dynamic compression 1 Hz with 10% sinusoidal strain, superimposed on a 10% static offset strain. Mechanical loading was performed 1h a day over 7 consecutive days. ***Biochemical Analysis.*** DNA content was measured spectrofluorometrically using Hoechst 33258. The amount of GAG in the scaffolds and medium was determined by the dimethylmethylene blue dye method. ***Gene Expression Analysis.*** Collagens type-I (COL1), type-II (COL2), type-X (COL10), aggrecan (AGG), proteoglycan4 (PRG4), osterix transcription factor (Sp7), transforming growth factor-β1 (TGFB1), and transforming growth factor-β3 (TGFB3) were investigated and compared to 18S ribosomal RNA as the endogenous control.

Results: Total GAG synthesized (scaffolds plus medium) was normalized to DNA content. The control samples cultured in medium with 1 ng/ml ($P<0.01$) or 10 ng/ml TGF-β1 ($P<0.001$) had significantly higher GAG/DNA value compared to samples cultured in medium without TGF-β1. In all the 3 groups where samples were cultured in medium with different concentrations of TGF-β1, the total GAG/DNA value showed a trend of up-regulation by mechanical load, this difference was significant in the groups with 0 ng/ml or 10 ng/ml TGF-β1.

As expected the addition of TGFβ led to an increase in chondrogenesis in a dose dependant manner. By day 14, in the absence of load, addition of 1 ng/ml TGF-β1 increased the COL2, AGG, COL10 and Sp7 gene expression of hMSCs 46392 ($P=0.004$), 687 ($P=0.002$), 60 ($P=0.002$) and 121 ($P=0.004$) fold respectively compared to cells cultured in the absence of TGF-β1. This increase was greater when 10 ng/ml TGF-β1 was added to the medium (Fig. 1).

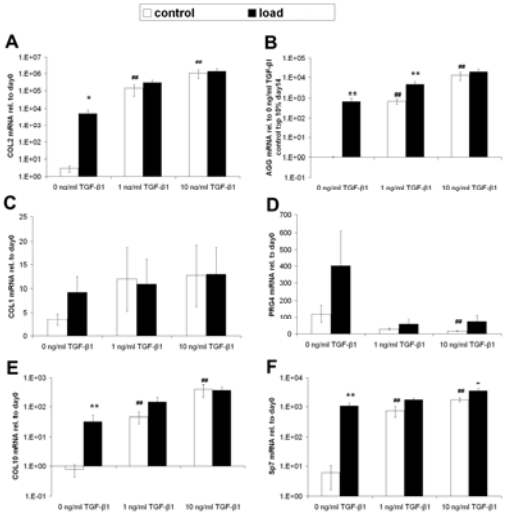

Fig 1. Effect of TGFβ and load on MSC gene expression

When investigating the effect of load on chondrogenesis increasing concentrations of TGFβ lead to a diminished response. The greatest response to load was seen in the groups without TGFβ. When hMSCs were cultured in medium without TGF-β1, mechanical load significantly stimulated gene expression by 1663 ($P=0.018$), 269 ($P=0.004$), 42 ($P=0.004$), and 174 ($P=0.006$) fold for COL2, AGG, COL10 and Sp7 respectively (Fig. 1). This suggests that under natural in vivo conditions mechanical load would be required to fully realise the chondrogenic potential of stem cells within this scaffold system.

Conclusions: This study demonstrates that the scaffold composite described is able to support chondrogenesis. The requirement for TGFβ in the medium can be removed when sufficient mechanical stimulation is applied. This study also shows that to more accurately determine the in-vivo response of a cell-biomaterial implant all stimuli, including mechanical load, must be considered.