Role of Shear and Compression in a Chondrogenesis In-Vitro Model

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INTRODUCTION: Cartilage defects present a unique clinical challenge due to their avascular nature. Mesenchymal Stromal Cells (MSCs) are multipotent cells located in many body tissues and are of particular interest due to their ability to repair cartilage tissue, via a process called chondrogenesis. In addition, in-vitro models have been recently focusing more and more on how MSC fate is affected by the application of a mechanical load. This can be done using bioreactor systems: depending on the design, different motions can be applied such as shear or compression forces, or even a combination. Using an established bioreactor chondrogenesis in-vitro model, it was previously shown that both shear and compression stimuli are necessary to trigger chondrogenesis in MSCs in the absence of exogenous Transforming Growth Factor Beta 1 (TGF-β1) [1]. Moreover, TGF-β1, an important growth factor involved in chondrogenesis and produced by cells in an inactive (latent) form, can be activated solely via mechanical load [2] and as a result, mechanical load modulates chondrogenesis via TGF-β1 pathway [3]. In this study, the role of shear and compression in terms of TGF-β1 activation and localization was investigated. Following this, a chondrogenesis study on the role of these two forces was conducted. The aim of this work is to identify the role of shear and compression in MSC chondrogenesis.

METHODS: Polyurethane (PU) foam scaffolds (8 mm diameter, 4 mm height) were filled with fibrin in the absence of cells, covered in DMEM containing 50 ng/mL human latent TGF-β1 and subjected to either shear load at increasing frequencies (0.01 Hz, 0.1 Hz, 1 Hz), compression at increasing strain (10%, 15%, 20%) or left in a free-swelling (FS) state, for 6 hours. For cell containing experiments, the scaffolds were filled with 5 million fibrin-embedded human MSC obtained with full ethical approval. The constructs were loaded for 15 days over a culture period of 22 days. Three different protocols were tested: P1 (1 hour of shear at 1 Hz followed by 1 hour of 20% compression), P2 (shear at 1Hz and 20% compression applied simultaneously for 2 hours), P3 (shear at 1Hz and 20% compression applied simultaneously for 1 hours) and compared to FS controls. Active TGF-β1, glycosaminoglycan (GAG), gene expression were quantified respectively with ELISA kit, DMMB assay, qRT-PCR. Safranin-O/FastGreen staining was performed on deparaffinized histological sections. Every test was taken at least in technical duplicates, for three different biological donors. For normally distributed data, one-way repeated measures ANOVA with post-hoc pairwise paired t-test was used; otherwise, Friedman test with post-hoc pairwise paired Wilcoxon test with Bonferroni adjustment was used, based on the Shapiro-Wilk test.

RESULTS: From the cell-free results, TGF-β1 is activated prevalently by shear forces and its concentration in the media increases with increasing shear frequency (Fig1a). Total (active and latent) TGF-β1 is more concentrated in the 20% compression treated scaffolds, meaning this compression pushed it into the scaffold that was initially not loaded with any TGF-β1 (Fig1b). For the cell-containing experiment, DNA content was not significantly altered by the load application. Similar levels of active endogenous TGF-β1 were measured for all the loading protocols. For all the donors, the SOX9/RUNX2 ratio is comparable or higher in all the three loaded groups than in the positive control (Fig2). The ratios of chondrocytic marker genes over fibroblastic marker gene (COL2/COL1, ACAN/COL1) and over hypertrophic marker gene (COL2/COL10, ACAN/COL10) are comparable with the positive control ratios for all the donors (Fig3). The data from the gene expression, GAG analysis and histological staining followed a similar trend. There were no statistical differences detected among the groups.

DISCUSSION: From the cell-free results, TGF-β1 activation seems to be mainly mediated by shear forces in the media. Compression is responsible for the fluid flow into the scaffold and therefore pushing the activated protein inside, especially at higher stains. From the cell-containing results, P1 decouples shear and compression, while P2 and P3 are time and cycle matched protocols with coupled forces. Therefore, during loading, in P1 the two forces are not affecting each other in their action on the system. The absence of statistical difference among the loading groups and their very similar trends in basically all the analysis show that the two forces are not affecting each other in their effect on cells and have a very defined role in this system, as described in the cell-free experiments.

SIGNIFICANCE/CLINICAL RELEVANCE: Movements that combine compression and bending of the joint in rehabilitation practice may be optimal for cartilage regeneration at the cellular level, as each seem to have their defined role on TGF-β activation and bioavailability. This might help to tailor the physiotherapy protocols for a better healing.

REFERENCES: [1] Schatti, O., et al, Eur Cell Mater, 2011 [2] Gardner, O.F.W., et al., J Tissue Eng Regen Med, 2017 [3] Li, Z., et al., J Cell Mol Med, 2010

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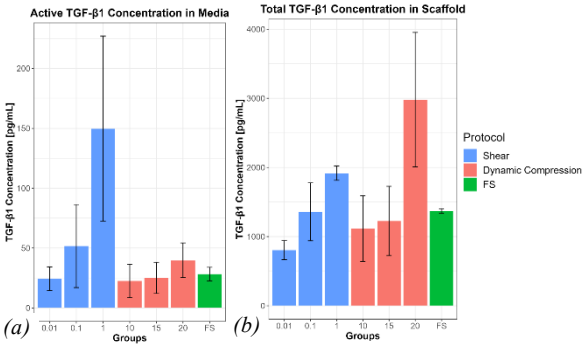

Figure 1: (a) Concentration of active TGF-β1 in the media (b) Concentration of total TGF-β1 inside the scaffold. Note: the graphs have different y scales.

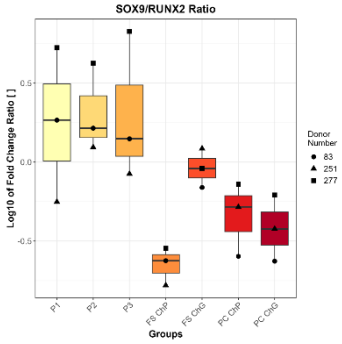

Figure 2: SOX9/RUNX2 ratio.

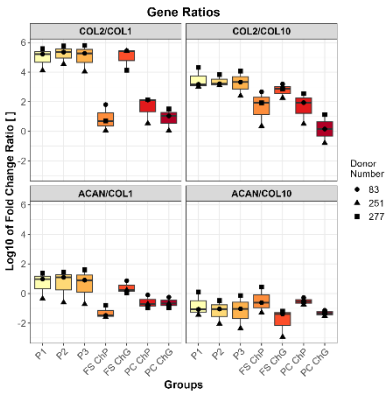

Figure 3: Gene ratios.