

# Finite Element Model Predicting Mechanical Latent TGF-β1 Activation in a Regenerative Cartilage Model

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**INTRODUCTION:** Articular cartilage defects present a unique clinical challenge, due to their avascular nature and consequent poor self-healing capacity. Cartilage regenerative models have become a necessary tool to better study and understand cellular healing mechanisms, to achieve more effective therapies for cartilage regeneration. Human Mesenchymal Stromal cells (hMSCs) are largely used in these models, due to their chondrogenic potential. Moreover, Transforming Growth Factor Beta 1 (TGF-β1) protein has a role in chondro-differentiation; it is produced in a latent form by the cells and in-vivo can be activated both via biochemical and biomechanical stimuli. In-vitro, bioreactors can provide mechanical stimuli that mimic the in-vivo forces experienced in cartilage tissue. Among the many different biomaterials that can be used to produce scaffolds supporting cells in in-vitro models, thermoplastic polyurethane (TPU) is an appealing candidate due to its biocompatibility and its printability in different structures and sizes. This allows the final mechanical properties of the structure to be tuned, according to what is of best interest. Additionally, finite element (FE) models are an effective tool to predict stresses and strains in a chosen structure and, once validated, could help optimizing the scaffold structure. In this study, as preliminary validation, we aim to correlate the activation of TGF-β1 protein with FE-predicted maximal principal strain in a bioreactor loaded scaffold.

**METHODS:** Scaffolds (Ø 8mm, 4 mm height) were punched out from a 3D-printed sheet of TPU, with 16.9% infill density and gyroid structure. They were then filled with cell-free fibrin, covered in media containing 50 ng/mL exogenous latent TGF-β1 and loaded in a mechanical bioreactor [1] for 6h with 10% preload plus 10%, 15% or 20% compression at 1 Hz. Unloaded controls were kept in the same incubator. ELISA DuoSet TGF-beta1 kit (R&D Systems) was used to quantify the protein concentration in the media and in the scaffold. Scaffolds were washed with RIPA buffer to allow recovery of the protein and its quantification. A 3D model of the scaffold was built in Simpleware (v.2017, Synopsys) and imported in Abaqus (v2021, Dassault Systems) to create an FE model of the bioreactor-scaffold system, where pure axial displacements corresponding to the specified strains above were simulated. Maximum principal strain [2] was calculated in the FE model within the fibrin region surrounding the 3D printed scaffold a median value was calculated at each applied strain step.

**RESULTS SECTION:** The concentration of active TGF-β1 protein inside the scaffold increased with the increasing compression state of loading, differently from the one measured in the media. The total protein is comparable for all the groups, for both scaffold and media. (Figure 1). Group differences were calculated using one-way ANOVA test, showing that the 10% and the 20% compression groups were statistically different ( $p < 0.05$ ;  $n=3$ ). In vitro, cells would be in the fibrin region where the FE-predicted maximum principal strain was higher due to the lower stiffness modulus (Figure 2). The values of active protein measured in the scaffolds at different loadings were correlated with the median of the maximum principal strain calculated from the FE model, resulting in a Pearson correlation coefficient of 0.986.

**DISCUSSION:** Preliminary results of the FE model shows the strain distribution inside the loaded scaffold, under different loading protocols. Under the assumption that the strain and stresses are constant over time, the bulk measures on the scaffolds confirm that higher levels of active TGF-β1 are found at higher strain levels. The high correlation coefficient (Figure 3) could be due to a low number of replicates, which need to be increased in future experiments. Nevertheless, all experiments were conducted in cell-free scaffolds ensuring that the action on the protein is fully mechanical and no biological processes are involved, which is captured by the FE model. Further work is ongoing to assess the spatial distribution of active TGF-β1 inside the scaffold and to correlate it with the local strains. Due to the shape of the scaffold the external compression load might result in local shear activating TGF-β1. The fluid flow around and in the scaffold contributes as well to the activation mechanism and its integration in the FEM cannot be neglected. Next, embedding of cells in fibrin will be necessary to give a more detailed picture of the biological model. The activation of TGF-β1 will be affected by the lower stiffness modulus of the mix fibrin gel-cells and this will be described in the FE simulation in terms of strains, as already shown for the cell-free scaffolds. Overall, a methodology to predict the potential of the scaffolds regarding the TGF-β1 activation has been developed and this can be potentially applied to any other scaffold structure to test it prior laboratory work. Other mechanically related biological features could also be investigated using the same approach.

**SIGNIFICANCE/CLINICAL RELEVANCE:** FE models can be of great help in preliminary test to assess if a biomaterial or scaffold structure is suitable to build a cartilage model for regenerative purposes. This could speed up the research and allow researchers to focus on understanding of regenerative processes and on testing of new treatments instead of spending too much time in the optimization of the biomaterial.

**REFERENCES:** [1] Gardner, O.F.W., et al., J Tissue Eng Regen Med, 2017. [2] Zahedmanesh, H., et al., Tissue Eng Part A, 2014.

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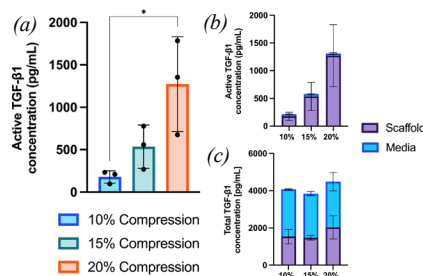


Figure 1: (a) Concentration of active TGF-β1 measured inside the scaffold. \*,  $p < 0.05$ . Concentration of (b) active and (c) total protein inside the scaffold and outside in the media.

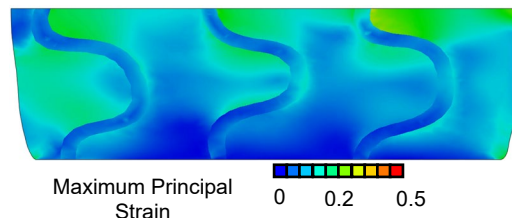


Figure 2: Contour plot of section view of FE model showing maximum principal strain (mm/mm) distribution in a section of the scaffold under the 20% strain loading scenario. Strain is seen to be higher in the fibrin region compared to the stiffer scaffold region.

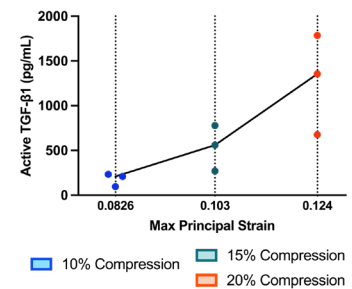


Figure 3: Correlation analysis between the values of active TGF-β1 and of maximum principal strain. Pearson coefficient,  $r=0.986$ .