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Mechanical stimulation of mesenchymal stem/stromal cells in a bioreactor system: An approach to mobilize cells into scaffolds

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Articular cartilage (AC) is a viscoelastic avascular tissue mainly composed of chondrocytes embedded in a rich extracellular matrix that bears and distributes loads occurring in the joints. The absence of vessels restricts its regenerative capability. Hence, joint motion facilitates nutrient deposition and cell waste disposal. Mechanical stimulation contributes to the homeostasis of functional AC by supporting delivery of nutrients, cytokines and growth factors between the distant chondrocytes. Current techniques to treat AC defects still fail to entirely heal and to achieve a native-like AC. Instead, a fibrous tissue with poor mechanical and biochemical properties is obtained. Since the knee joint has neighboring niches of stem cells, we hypothesized that mechanical stimulation might enhance the mobilization of endogenous mesenchymal stem/stromal cells (MSCs) from nearby niches as the bone marrow (BM).

This study aimed to introduce a novel bioreactor system *in vitro*, capable of inducing dynamic mechanical loading on a scaffold; and evaluate whether MSCs could be mobilized from a compartment beneath to a scaffold after the mechanical stimulation, as cells might move when the subchondral bone is opened. This was a risky approach, as there are little evidences existing to base our assumption on; and both, the bioreactor as well as the experimental setup (including efficient cell characterization in 3D) had to be developed, optimized and then finally evaluated.

A novel mechanical system for evaluating mobilization of cells in a 3D context *in vitro* is presented. The system consists of a) a compression bioreactor able to induce loading on scaffolds, b) custom-made software for settings for management and data recording, c) cell loading experiments and d) 3D image-based biological evaluation.

The mechanical stimulation acted on an acellular scaffold made of alginate, functionalized-alginate or collagen, and a cell reservoir containing porcine or human BM-MSCs (pBM-MSCs and hBM-MSCs, respectively) below it. The mechanical loading program was set up as 10 % strain regarding the original height of the scaffold, 24 hours at 0.3 Hz, under dynamic continuous or intermittent regime, with unload phases of 10 seconds each 180 cycles when intermittent loading was used.

Supporting our hypothesis, intermittent mechanical stimulation induced the mobilization of hBM-MSCs in collagen scaffolds 10-fold compared to the unloaded control, as well as pBM-MSCs mobilized 4-fold in functionalized-alginate scaffold, when intermittently loaded. Remarkably, the viability of mobilized cells was not compromised by intermittent mechanical loading application as evaluated under an optimized and validated protocol for counting and viability cell detection in 3D. In addition, we found that the bioreactor was able to stimulate the scaffolds and the cells for 23.09 ± 0.94 hours in 137.72 ± 13.21 periods, exerting compression with vertical piston displacements of 230.08 ± 54.07 µm, force of 1.08 ± 0.13 N for hBM-MSCs and force-amplitude of 1.86 ± 1.46 N for pBM-MSCs.

In this study, a bioreactor system comprising unique hardware and software architecture, separated devices for cell cultivation, mechanical application, and software was optimized to evaluate the role of mechanical stimulation on mobilizing MSCs toward scaffolds *in vitro*. The bioreactor system worked well as it was able to provide mechanical stimulation over the scaffolds. Remarkably, intermittent mechanical stimulation induced the mobilization of viable pBM-MSCs into functionalized-alginate and hBM-MSCs collagen scaffolds. The cells were mobilized from a lower compartment of the bioreactor toward the scaffolds in another compartment above, against gravity.

As a first step to induce cartilage regeneration *in situ*, this study provides a tool to enrich acellular scaffolds with viable MSCs after mechanical stimulation. Thus, the applicability of these findings for the orthopedic research field is to establish a biomechanical system *in vitro* with the possibility to use mechanical stimulation on cells moving from a compartment beneath, simulating MSCs moving *in vivo* from bone marrow into the cartilage defect in a knee joint. This experimental approach might be used in

the future to study molecular factors of mobilized and non-mobilized cells that help to identify further biochemical or mechanical agents for recruiting cells *in vitro*. Further studies need to be done to address whether cartilage regeneration can be done using the mobilized MSCs. A strategy that combines biomechanical protocols and functionalized scaffolds, as the presented here, with current strategies already used for AC-regeneration as microfracture might contribute to a better outcome of the current treatments applied in osteoarthritis or AC-trauma.

Derived from this study, we were able to apply for a Model Utility Protection Patent, we published an original paper and we also had the opportunity to communicate the developed methods and interesting findings in oral and poster presentations.

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