Mechanical Dissipation As A New Variable For Cartilage Mechanobiology

Philippe Abdel-Sayed, Salim Darwiche, Dominique Pioletti.
EPFL, Lausanne, Switzerland.

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Introduction: Mechanical stimulation has been demonstrated to be one of the strategies to optimize chondrogenesis and improve the properties of the cell-based constructs. Nevertheless, we are still far from completely understanding the intricacies involved in the influence of scaffold structure on cells and the cell-matrix interaction. This makes it particularly challenging to rationally determine the optimal mechanical stimulation to control chondrogenesis. For instance, Li and colleagues have shown that the parameters of frequency and amplitude during dynamic compression modulate chondrogenesis of human bone marrow mesenchymal stem cells seeded in polymeric scaffolds [1]. However, particular frequency and amplitude may be optimal for one type of scaffold but may not be for another type, since the scaffolds would differ in their mechanical properties and in their microstructure, inducing then a different stimulus to the cells. This leads to the need of identifying an overarching variable to generally take into account those parameters and to be relevant to cartilage microstructure and function. Dissipation is one of the characteristics of viscoelastic materials such as cartilage and polymeric scaffolds. It can be modulated by the above-mentioned parameters and is relevant to the microstructure and function of cartilage and biomaterials. Hence, the aim of this study is to assess the effect of energy dissipation of polymeric scaffolds on chondrogenesis.

Methods: Cell-scaffolds construct preparation: Different HEMA-based scaffolds have been developed having different levels of dissipation when subject to the same loading in terms of frequency and amplitude of deformation. The levels of dissipation where chosen to match the level of dissipation of healthy and degenerated cartilage. Briefly, salt leaching method has been used to fabricate scaffolds containing 4%, 6%, 8% and 10% ethylene glycol dimethacrylate used as crosslinker. All the scaffolds were coated with fibronectin in order to uniform the cell-scaffold interface between the different types of scaffold. Scaffolds were then seeded with epiphyseal chondro-progenitor cells (ECPs) [2] at 3 mio cells/scaffold. Scaffolds characterization: Mechanical properties. The mechanical properties of the scaffolds were measured in terms of energy dissipation and dynamic stiffness. Unconfined compression tests were made using an Electropuls Dynamic Test System on cylindrical specimens immersed in deionized water at RT placed in a custom-made set-up. Sinusoidal compressive loading of 10% amplitude (10% pre-strain) was applied at a frequency of 1 Hz. The dissipation was calculated from the load-displacement graphs as the integral of the area enclosed by the hysteresis curve. The dynamic stiffness was measured as the slope between the maximum force-displacement and the minimum force-displacement. The contribution of the fluid-phase to the mechanical properties was measured by reducing the fluid content in scaffolds. Hence, we defined the fully saturated state of the scaffolds as the scaffolds swelled to equilibrium. We defined the unsaturated state as the saturated scaffolds putted under vacuum 10 minutes to remove partially water content, and dry scaffolds as the scaffolds dried at 100°C for 2 h after the vacuum process. Scaffold architecture. Scaffold morphologies were determined by micro-computed tomography. Surface topographic features including pores, pillars, or grooves that may influence cellular behavior were determined by scanning electron microscopy. Cell viability and adhesion. The LIVE/DEAD staining kit (Invitrogen) was used to quantify live and dead cells up to 28 days after seeding of ECPs. Immunostaining of actin filament and fibronectin was used to verify cell adhesion on scaffolds after 4 days of culture.

Chondrogenic expression subsequent to mechanical stimulation: Cell-scaffold constructs of the different groups were subject to mechanical stimulation. Dynamic compression of 1 Hz and 10% strain (with 10% pre-strain) was applied to scaffolds 2 h/day during 4 consecutive days. After the 4th day of testing, immediately after mechanical stimulation, gene expressions of TGF-beta, Sox9, Col2a and Aggrecan were analyzed for the loading group and the free-swelling group used as control. Statistics: Data are expressed as mean ± SD (n=5 per group of scaffolds).

Results: Dynamic compression of the scaffolds showed that the dissipation is higher for scaffolds with higher amount of crosslinker, given a same mechanical stimulation (Fig. 1.a). This dissipation is fluid-depend for 6%, 8% and 10% as it may be observed that reducing the water content also reduced the level of dissipation, because of lower stiffness due to fluid pressurization (Fig. 1.b). The different groups of scaffolds were scanned by micro-computed tomography. The results showed a good interconnectivity between the pores with 67% porosity (Fig. 2a). The distributions of pore size and structure thickness were the same for the different groups of scaffold. The mean pore size was 147 μm and the mean structure size was 160 μm. SEM images showed that the surface topology is the same between the different group of scaffolds, and no apparent micro-porosity was present (Fig. 2b). Cell viability assay after 28 days showed good viability for all the different groups (Fig. 3a).

Immunohistochemistry performed on fibronectin-coated scaffolds showed homogeneous distribution of the fibronectin allover the scaffold surface, and good stretching of the actin fiber, relevant to a good adhesion to the scaffold surface (Fig. 3b). In order to assess the effect of mechanical dissipation on cell behavior, cell-scaffolds constructs were subject to dynamic compression 2 hours/day during 4 days. The profiles for gene expressions (TGF-beta3, Sox9, Col2a, Aggrecan) after the 4 days are shown in Fig.
4. In general, upregulation for all genes is observed with dissipation close to the dissipation of healthy cartilage, and downregulation is observed for dissipation close to degenerated cartilage.

**Discussion:** The goal of this study was to evaluate dissipation as mechanobiological variable affecting chondrogenic expression. ECPs were subject to unconfined compression 2h/day during 4 days in scaffolds having different dissipation under same loading. Higher dissipation with fluid-flow component had upregulated chondrogenic markers. Effect of dissipation in mechanobiology has already been observed for bone, where cellular response of mouse bones was maximal when the dissipation was maximal [3]. Likewise, it has been proposed that bone remodeling may be based on energy dissipation [4]. The results of this study suggest that energy dissipation may play a role in cartilage mechanobiology.

**Significance:** We have shown the sensitivity of chondrogenic expression to dissipation, presenting it as a new variable to be considered in cartilage mechanobiology. It can be proposed to use dissipation to optimize bioreactor systems.

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**References:**
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**Fig. 1.** (a) Energy dissipation of scaffolds and their corresponding dynamic stiffness (b). The green lines correspond to the values for healthy normal human cartilage, and in red the values for degenerated cartilage. For the dynamic stiffness the values of cartilage were above the values of the scaffolds (160 and 80 N/mm respectively).

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**Fig. 2.** (a) Image of a reconstructed poly(HEMA-co-EGDMA) scaffold from uCT scans showing the pores interconnectivity. (b) SEM image.

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**Fig. 3.** (a) Cell viability in scaffold. In green, live cells (b) Immunohistochemistry of ECP cells seeded on coated-scaffold. In green the actin fibers and in red
fibronectin. Scale bar = 100 um.

Fig. 4. Gene expression graphs for (a) TGF-beta 3, (b) Sox9, (c) Col2 and (d) Acan.

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