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Chondrocyte migration in an acellular tissue-engineered cartilage substitute

Julia Nachtsheim[1], **Gözde Dursun**[1], **Bernd Markert**[1], and **Marcus Stoffel**[1,*]

[1] Institute of General Mechanics, RWTH Aachen University, Templergraben 64, 52062 Aachen, Germany

Acellular tissue-engineered cartilage substitutes are promising replacement materials to mend small focal defects. The underlying therapeutic idea is to initially stabilise the defect zone and in a long-term manner allowing for remodelling processes towards a hyaline cartilage tissue. Crucial for remodelling processes inside the implant, is the cell migration from the adjacent tissue, which will be assessed in this study.

1 Introduction

Small local articular cartilage defects due to traumata or diseases require an early treatment to prevent the development of a subsequent osteoarthritis [1]. A new therapeutic approach to mend small defects is the implantation of a cell-free collagen matrix. The hydrated collagen scaffold is composed of collagen type-I and can be minimal-invasively inserted as a plug or casted into the defect zone. Once implanted, the scaffold immediately provides mechanical stability to the tissue. In the long term, the tissue allows for remodelling processes aiming to restore an articular cartilage construct. First clinical studies have revealed promising results showing a significant improvement of the afflictions one year after surgery, a very good integration into the surrounding cartilage tissue, and have identifying cells inside the implant [2, 3]. Cell immigration from the adjacent tissue, from the underlying bone or from the synovial fluid, is essential for inducing cellular remodelling processes inside the tissue and, hence, for the therapeutic success. Cell migration is a complex process, which is influenced by various factors, such as composition and structure of the scaffold or a chemical or mechanical stimulus. Addressing some fundamental questions of chondrocyte migration, we here introduce an *in vitro* model to assess chondrocyte migration in different collagen matrices including commercially available medical products and, furthermore, we analyse the influence of a mechanical stimulus.

2 Materials and methods

2.1 In vitro model

An in vivo model is used to analyse cell migration. A thin layer of human chondrocyte seeded collagen type I is prepared and one of each implants is positioned on top. Collagen type 1 matrices with a concentration of 6 mg/mL and 8 mg/mL are used as implants and compared to two commercially available implants, ChondroFiller gel, and gelled plugs of ChondroFiller liquid (Amedrix GmbH, Esslingen, Germany). These constructs are then either cultured statically or dynamically for a period of twenty days. Then, Life Dead stain is performed and samples are microscopically analysed with a confocal microscope. Using image processing software fiji (v1.52d) [4], each cell can be allocated a position inside the implant and the cell distribution inside the implant can be evaluated.

2.2 Dynamic cultivation

Samples are dynamically cultivated in an in-house made bioreactor system as shown in figure 1a. The fully immersed constructs are positioned in a sterile chamber, which is restricted by a membrane at the bottom. The load cell is positioned underneath the membrane recording the reactive forces. Mechanical stimulation is performed displacement controlled by a stepper motor, which transmits the vertical movement to a solid and flat-ended stamp. A more detailed description of the bioreactor system can be found in [5].
One stimulation cycle includes four stimulation intervals of 30 minutes each ($\epsilon = 5\%$, $f = 0.5$ Hz) and pauses of 60 minutes. During the total cultivation of 20 days, samples were alternate resting for two days and stimulated for four days. All four collagen matrices feature a very soft and highly viscous material behaviour. If a cyclic strain with an amplitude of 5 % is applied, the amplitude of the reaction force will be in the range of 12 mN.

* Corresponding author: e-mail stoffel@iam.rwth-aachen.de, phone +49 241 80 94589, fax +49 241 80 92231

3 Experimental results

Figure 1 shows the experimental results of the cell migration experiment. Chondrocytes have migrated from the cell-seeded collagen matrix into all four adjacent implants when cultured statically or dynamically. Cell migration inside the ChondroFiller gel under dynamic culturing conditions was not assessed after four weeks, but cells have been observed inside the implant during routine microscopy.

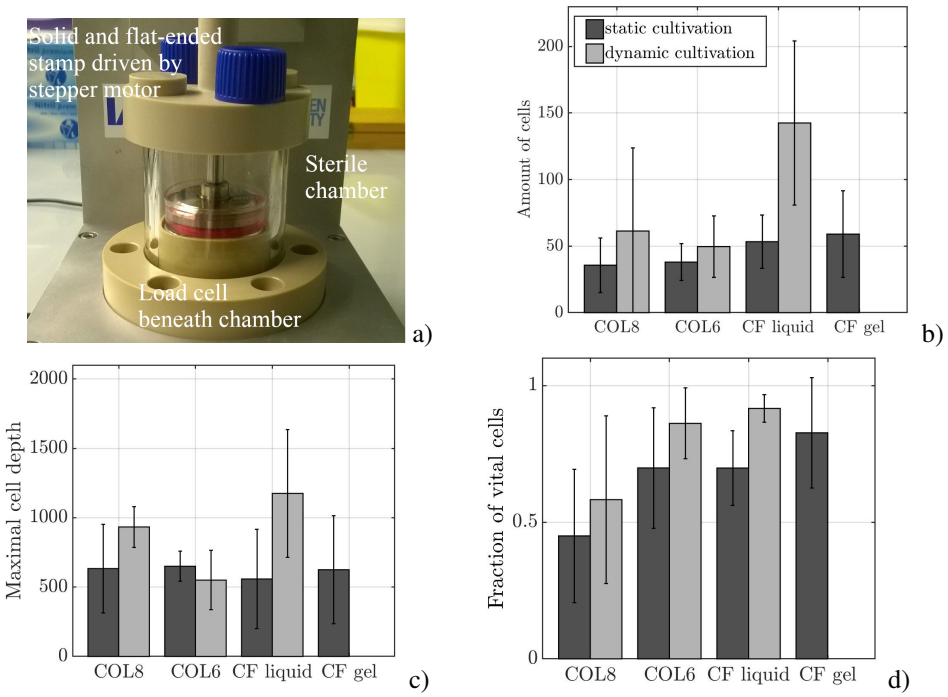

Fig. 1: In-house compression bioreactor system **a**, total amount of cells inside the implant **b**, fraction of vital cells **c**, maximal migration depth in μm **d**. All error bars represent the standard deviation.

The total amount of cells inside the implant is higher when cultivated dynamically. There are two underlying effects, which cannot be distinguished in the frame of this study. First of all, the mechanical stimulus induces cell proliferation, which will increase the total amount of cells. In the mean time, the mechanical stimulus might enhance the cell movement. However, clinical motivation focusses on the therapeutic success, therefore, it is not neccessary to distinguish between cell proliferation and cell migration.
A significant increase of the amount of cells inside the implant can be observed with the product ChondroFiller gel, also featuring the largest difference in maximal cell depth. An increase of this measure does not apply for all four implants. This depth is a minor characteristic for the actual distance moved by the cell, since the migration path of a cell is not restricted to one direction and the experimental setup does not allow for tracking the movement of a single cell.

4 Conclusion

In vitro models are an important feature to simulate and analyse *in vivo* processes. We have here introduced an *in vitro* model to assess chondrocyte migration from an adjacent tissue into an accellular collagen matrix, which is clinically used as a cartilage replacement material. Cell migration has been analysed in a static and in a dynamic culture condition. To stimulate the constructs dynamically, a physiological compressive load is periodically applied using an in-house bioreactor system. For collagen implants of different concentrations and for commercially available medical products chondrocyte migration into the implants was observed and the total amount of cells inside the implant was increased due to the dynamic stimulus.

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