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Experimental Study on Cell-free Approach for Articular Cartilage Treatment

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Abstract: Cell-free based approaches are introduced as a promising treatment method for articular cartilage. The success of this method requires cell colonisation from resident tissue into cell-free implants. The objective of our study is to promote the cell colonisation into cell-free collagen I based implants by mechanical stimulation. Therefore, a new *in vitro* cellular model consisting chondrocyte-seeded matrix and cell-free implants was developed in a polydimethylsiloxan (PDMS) mold. These constructs were cultured under dynamic and static culture conditions. For the dynamic culture, we have developed an in-house bioreactor system where both the load and the deformation applied to the specimen are recorded. Cyclic compression, with a strain of 5% and frequency of 0.1 Hz, was applied to constructs without any break. At the end of three days of dynamic and static cultivation, the cell-free implants were seperated from cell-seeded matrix and cultured in a petri dish three days long. Afterwards, they were analysed using fluoresence dyes. The microscopic assessment indicated that there was a cell migration into the cell-free implants which were cultured dynamically.

Keywords: Articular cartilage, cell-free implants, collagen, chondrocyte migration

1 Introduction

Articular cartilage repair and regeneration still remains challenging in clinical applications since the self-repair capacity is limited due to the avascular nature of articular cartilage [1]. Recently, the use of tissue-engineered articular cartilage constructs has introduced a promising treatment option. However, the clinical translation of tissue engineering is not only time-consuming but also has some other drawbacks such as lack of cell sources, possible host immune complications and difficult preservation and transportation [2]. In order to overcome these limitations, cell-free based approaches leading to *in situ* tissue regeneration have been offered as a one-step procedure. The success of this new therapeutic approach requires cell colonisation into cell-free implants. Besides, biochemical and biomechanical stimulation acting on the target therapeutic cells play significant roles by promoting cellular processes [3][4]. Previous studies have already shown the potential of cell-free collagen type-I implants to trigger the cellular in-growth and create a good quality repair tissue in short and long terms [5][6]. However, based on the knowledge of authors, there are no studies applying mechanical stimulation to promote and accelerate the chondrocyte migration into the cell-free implants [7][8].

The objective of this study is to assess chondrocyte migration into the cell-free collagen implants under static and dynamic conditions. In order to create a dynamic culture condition, we have developed an in-house bioreactor system which is capable of applying mechanical strain to cellular constructs in a tightly controlled and monitored environment. Using this system, cell-free collagen implants are cultivated in contact with a matrix seeded with chondrocytes under uniaxial loading regime. We have further investigated the effect of applied mechanical stimulation on the chondrocyte colonisation into the cell-free implants. Our results demonstrate that uniaxial cyclic compression promotes the cell colonisation in a short term of cultivation.

2 Materials and Methods

2.1 Experimental design

The *in vitro* cellular model was prepared in a polydimethylsiloxan (PDMS) mold placed in petri dish. PDMS mold possesses seven wells allowing the parallel stimulation and cell cultivation. The first layer of *in vitro* cellular model was prepared with a matrix (PL Bioscience, Aachen, Germany) and human articular chondrocytes (Promocell, Heidelberg, Germany) in passage three. The matrix provides three-dimensional (3D) cell culture system based on human platelet lysate which supplies nutrients to the cells. Firstly, 100 µl of matrix was pipetted into the wells of PDMS mold. After one-hour of gelation process, the harvested articular chondrocytes with the cell number of 45 000 cells/cm^2 were seeded on top of the matrix. 3D collagen type I based cell-free implants (Meidrix Biomaterials GmbH, Esslingen, Germany) were fabricated by mixing an acidic collagen solution of 10 mg/mL and a gel neutralisation solution in a ratio of 4:1. The diluted col-

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lagen solution of 8 mg/mL was cast into a cylindrical shape, with an 8 mm diameter and a 5 mm height. After the gelation process, the implants were brought into contact with the cell-seeded matrix (see Figure 1). The dynamically cultured *in vitro* model was put inside the compression bioreactor which was placed in an incubator with 5% CO_2 at 37°C while the statically cultured *in vitro* model was kept only in the incubator.

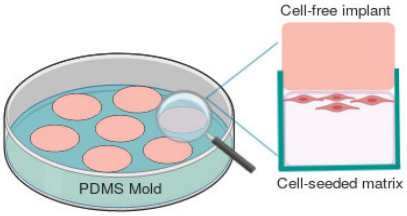

Fig. 1: Schematic picture of PDMS mold including chondrocyte-seeded matrix and cell-free collagen I based implants.

2.2 Mechanical stimulation in a compression bioreactor

The in-house compression bioreactor is capable of applying uniaxial mechanical strain controlled by the stepper motor. A flat piston positioned between the stepper motor and the cultivation chamber implements static or dynamic compression directly to the *in vitro* cellular constructs. The load cell placed under the cultivation chamber records the force during experiments. The cultivation chamber including the cell-seeded matrix and the cell-free implants was manufactured from autoclavable materials (PEEK(Polyether ether ketone) and glass) and possesses two filter caps for gas exchange [8] (see Figure 2).

Cyclic compression, with a strain of 5% and frequency of 0.1 Hz, was applied to dynamically cultured constructs without any break while statically cultured constructs were not exposed to any cyclic compression. After three days of dynamic cultivation and static cultivation, the cell-free implants which were separated from cell-seeded matrix were cultured in a petri dish fully immersed in the culture media for three days.

2.3 Imaging of cell migration

Cell-free implants were analysed using the Live/Dead cell assay which stains the live cells green and the dead cells red. At the end of the experiment period, specimens were washed in phosphate-buffered saline (PBS) (Gibco by Thermo Fisher Scientific, Germany) at room temperature, followed by incubation with 5 µM Calcein AM (Thermo Fisher Scientific, Germany) for 30 minutes and 5 µM PI (Thermo Fisher Scientific, Germany) for 10 minutes. Cell colonisation into the cell-free implants were monitored by taking pictures with inverted fluorescence phase contrast microscope (Keyence BZ 8100, Japan). A z-stack with the thickness of 1 µm was collected for each specimen in order to determine the maximal depth of cell migration in the implants.

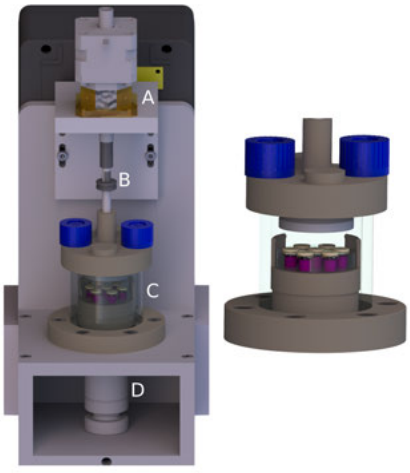

Fig. 2: Technical drawing of the in-house bioreactor system.(A) Stepper motor. (B) Flat piston. (C) Cultivation chamber. (D) Load cell (Left). The cultivation chamber including the *in vitro* cellular model (Right)

3 Results

A total of three cell-free implants were cultured under dynamic and static culture conditions. The microscopic assessment of dynamically cultured cell-free implants revealed that the chondrocytes migrates from the underneath cell-seeded matrix into the cell-free implants. In these implants, alive chondrocytes have been identified in green colour inside the cell-free implants while no dead chondrocytes were detected (see Figure 3).

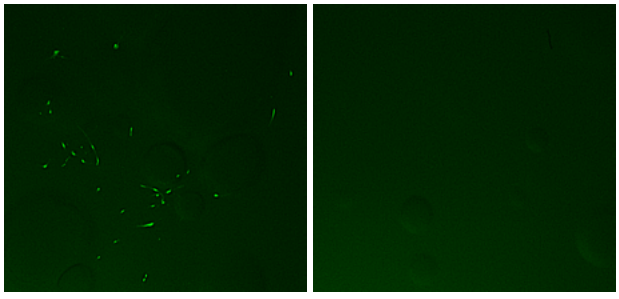

Fig. 3: Fluorescence microscope pictures of dynamically cultured (left) and statically cultured(right) cell-free collagen I based implants at a 2-fold magnification.

Conversely, there was neither alive nor dead chondrocytes observed in the statically cultured cell-free implants (see Figure 3). This result has strengthened our hypothesis that mechanical stimulation in the form of compression induces the chondrocyte migration into cell-free scaffolds.

The elongated cell morphology of the chondrocytes observed in dynamically cultured cell-free implants suggests, the cells were not sucked up into the implants mechanically, but rather entered by cell migration process (see Figure 4).

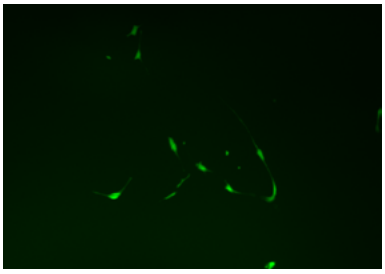

Fig. 4: Cellular shape of chondrocytes inside the cell-free collagen I based implants at a 10-fold magnification.

The z-stacks indicate that the chondrocytes were not just attached on the surface of implants, but they were located in different z-planes within the implants. As it is presented in Figure 5, the cells seen sharp on the top image slice of z-stack were blurry on the bottom image slice, and vice versa. The maximal depth of cell migration was 58 µm.

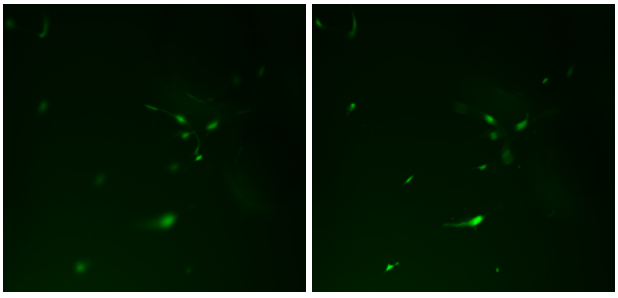

Fig. 5: The top(left) and the bottom(right) image slices of z-stacks.

4 Discussion

In vitro models play a key role in studying cellular processes [8]. In this study, we have established a new *in vitro* cellular model consisting of chondrocyte-seeded matrix and cell-free collagen I based implants in a PDMS mold to study the chondrocyte colonisation into cell-free implants. The advantage of this construct is that the PDMS mold surrounding the *in vitro* cellular model holds cell-seeded matrix and cell-free implants together during the experiments. Furthermore, based on the observation during cultivation, it was seen that the chondrocytes were spread into the matrix during experiments although they were only seeded on the matrix. This indicates that the matrix provides three-dimensional environment to the chondrocytes which dedifferentiate into fibroblast-like cells in monolayer culture [9].

Previous studies and clinical reports have demonstrated the presence of cells in the cell-free collagen I based implants. However, the source of colonizing cells has remained unclear [5][6]. In order to determine whether the source is the underlying cartilage tissue, we have here examined the migration potential of chondrocytes *in vitro*. Our results show that the chondrocytes migrate from the matrix into the cell-free implants. This confirms previous findings in the literature [8][10][11]. However, a number of questions concerning cell phenotype and function remain to be answered. The microscopic assessment and z-stacks indicated the residence of the chondrocytes in three-dimensional environment, but gene expression analysis of cartilage-specific genes such as type-II collagen, proteoglycan, aggrecan or collagen IX need to be determined in further experiments.

It is not yet well investigated whether the mechanical stimulation could promote and accelerate chondrocyte migration into the cell-free implants. The aim of the research was therefore to investigate the effect of mechanical stimulation on induction of chondrocyte migration. The microscopic assessment of cell-free implants indicated that there was a cell migration into the cell-free implants which were cultured in the compression bioreactor with a strain of 5% and frequency of 0.1 Hz for three days without any break. This confirms our previous experiments performed four times for 30 minutes mechanical stimulation with a strain of 5% and frequency of 0.5 Hz for 20 days [8]. Visibly, the cell number in the cell-free implants was far less than previous experiments [8] because of the shorter experiment duration. However, we have shown here that the short term mechanical stimulation also induces the chondrocyte colonisation process into the cell-free implants.

5 Conclusion

Cell-free based approaches have been introduced as a one-step treatment method for articular cartilage repair. This paper is a preliminary attempt to study the effect of mechanical stimulation on the chondrocyte colonisation which is a key step for the success of cell-free based approaches. We have therefore established an *in vitro* cellular model and applied a cyclic compression to this construct. Future work will focus on the optimisation of mechanical stimulation and the development of in-house compression bioreactor which enables more parallel cultivation of *in vitro* cellular model.

Author Statement

Research funding: The authors state no funding involved. Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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