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Bioreactor design for the mechanical stimulation by compression of 3D cell cultures

Abstract: Bioreactors with a controlled physiological environment are being developed to study various cell processes. The influences of mechanostimulation on bone cell cultures can be investigated using a compression bioreactor.
The developed bioreactor system applies a cyclic compression force to the specimen via an eccentrically mounted push rod. The compression force is monitored by a force sensor to detect changes in the material properties of the specimen. Depending on the piston setting, a stroke of 0.28 - 2.50 mm can be applied to the specimen.
The bioreactor system was tested with a trial run of 18 days. A sample was continuously stimulated with a loading frequency of 2 Hz and a stroke of 1.50 mm. The sterility in the cell chamber as well as the functionality of the realised bioreactor stimulation system could be successfully confirmed.

Keywords: Bioreactor, Compression, Tissue Engineering, 3D Cell Culture, Bone Cell Culture, Osteoblasts.

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1 Introduction

Culturing human cells plays an important role in regenerative medicine by helping to understand tissue formation and regeneration after trauma.
Cell cultivation takes place in vitro and can be performed in two or three dimensions, as well as with or without a scaffold. However, three-dimensional cell cultures are usually more clinically relevant as they behave more similar to in vivo than two-dimensional cell models [1,2]. Some cell processes can also only form in a three-dimensional structure [3,4].
With respect to bone tissue, the mechanisms and influences of mechanostimulation on cell behaviour are not yet sufficiently understood. Mechanical loading of the musculoskeletal system is one of the most important factors for bone growth and formation in vivo [5,6]. Therefore, understanding bone metabolism is essential for the restoration of traumatised bone tissue in regenerative medicine. Mechanical compression of a matrix structure cellularised with primary human osteoblasts is a promising approach to study the effects of mechanical stimulation on bone tissue [7,8]. For the cultivation and

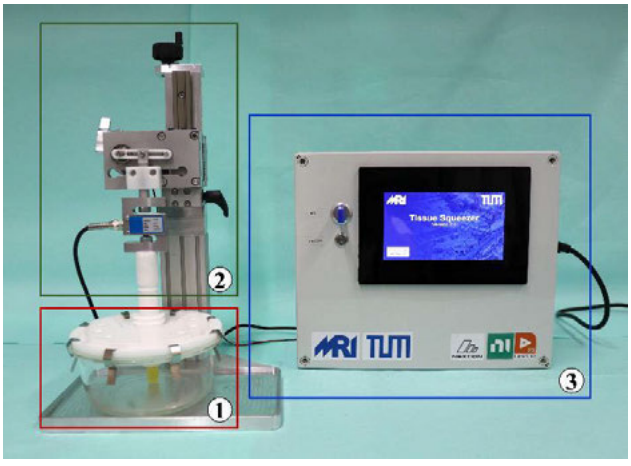

Figure 1: The bioreactor system includes 1) a cell chamber, 2) a mechanical stimulation unit for the compression force and 3) a control unit.

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mechanical stimulation of the bone cell culture a newly developed bioreactor with a controlled compression and physiological environment is necessary.

2 Methods

The designed compression bioreactor can be structured into three parts: the cell chamber, the mechanical stimulation unit, and the control unit (Fig. 1).

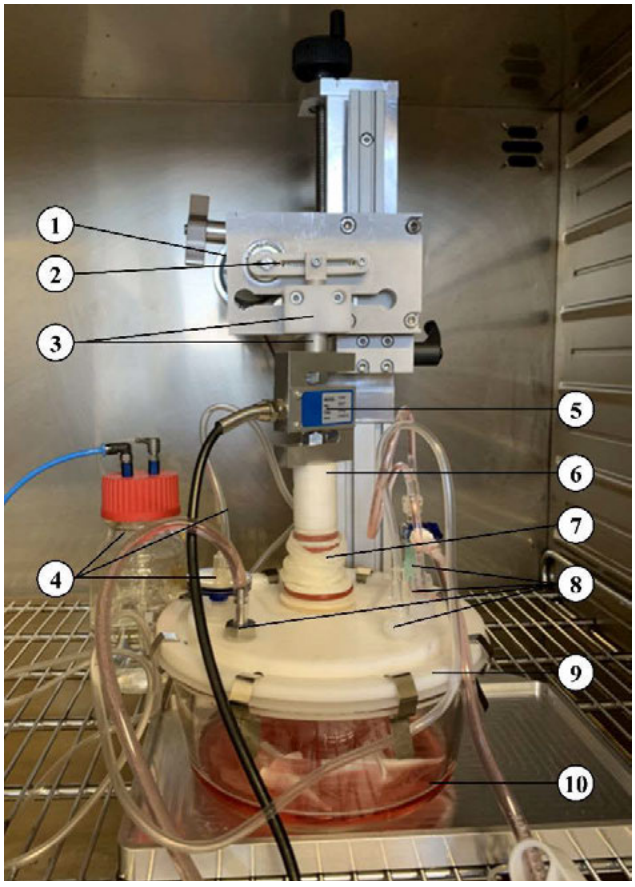

Figure 2: Cell chamber and mechanical stimulation unit of the bioreactor system: 1) DC Motor; 2) Eccentrically controlled push rod; 3) Guided piston; 4) CO_2 supply and pressure compensation; 5) S-type load cell; 6) Compression stamp; 7) Sterile interface with condom and O-rings; 8) Connections for medium in- and outflow; 9) Custom-made lid with clip mechanism; 10) cell chamber with DMEM.

2.1 Cell chamber

The central component of the bioreactor system is the cell chamber (Fig. 2), which must fulfill the requirements of sterility and biocompatibility. Due to this, the cell chamber and its components consist of autoclavable or sterile disposable products. A commercially available glass bowl from the household sector (borosilicate glass) is used which is supplemented with a custom-made lid. A clip mechanism is implemented as closing mechanism for the lid, which seals with an additional silicone ring. The lid, made of polyoxymethylene (POM), contains the corresponding connections for the nutrient supply of the specimen. The connections are provided on the one hand for CO_2 supply and pressure compensation and on the other hand for medium supply and discharge as well as for the medium change. The fluid flow of the culture medium is supported by an externally provided peristaltic pump (Arthrex Continuous Wave II Arthroscopy Pump AR-6450, Germany). The tubing of the fluid system must be changed after approximately one week, when the flexible tube section in the peristaltic pump shows the first signs of use and should not tear. The tubing change is possible via closable interfaces to the cell chamber, so that the sterile cell chamber cannot be contaminated during the change. In addition, another interface for the compression stamp is necessary, which is sealed by a dry condom (no lubricant) and O-rings. Despite the many interfaces, the cell chamber should stay closed and tight.

2.2 Mechanical stimulation unit

The mechanical stimulation unit applies a cyclic compressive force to the cell culture via a stamp made of POM (Fig. 2, 3). The cyclic compression on the cell construct is applied by a DC motor (Modelcraft DC Motor 50:1 12 V, Germany) with eccentrically controlled push rod and guided piston. This allows the rotary movement to be transferred into a linear movement. A deformation or rather stroke of 0.28 - 2.50 mm

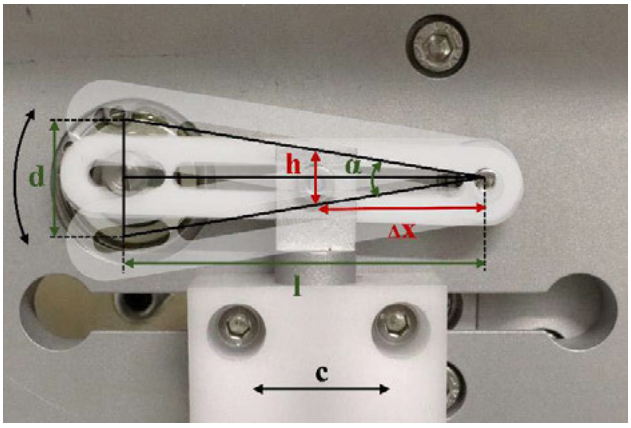

Figure 3: The DC motor and the eccentrically mounted push rod generate the stroke (h) on the piston, which can be varied by the displaceable mounted piston (translation c).

can be applied to the specimens by the displaceable mounted piston in the push rod (Fig. 3).

$$h = 2 \cdot \left(\tan\left(\frac{\alpha}{2}\right) \cdot \Delta x\right) \quad (1)$$

$$with\ \alpha = 2 \cdot \tan^{-1}\left(\frac{d}{2l}\right) \quad (2)$$

The position of the compression stamp is controlled by a forked light barrier (Vishay TCST 2103, USA) so that no compression force is applied during pauses in stimulation and the stamp is in its uppermost position. The compression force is monitored by a force sensor (S-type load cell CZL 302, Phidgets Inc., Canada) during the test. The implemented force sensor has a measuring range of 20 kg and a precision of 6 g. The sensor can detect changes in the material properties of the specimen and determine whether the cell construct has become stiffer.

2.3 Control unit

The bioreactor is designed as an automated, software-controlled system for mechanical stimulation with compression. The real-time capable controller myRIO from National Instruments (NI, USA), which is programmed via the LabVIEW (NI, USA) development environment, is used for control of the bioreactor. The real-time controller enables a standardized, 24/7 long-term cultivation over weeks. For documentation, the experimental data of the force sensor and the temperature sensor at the cell chamber are stored on a flash drive.

The custom experiment configuration and visual monitoring of the experiments is done by a 7'' resistive touch display (Nextion Intelligent Model NX8048P070, ITEAD Studio, China). The graphical user interface is designed for clear and intuitive operation and communicates via serial interface with the controller (Fig. 1).

2.4 Function test

The bioreactor system was tested with a technical trial run of 18 days. A dummy sample (foam) was continuously stimulated with a loading frequency of 2 Hz and a stroke of 1.5 mm. The stimulation system was placed in a heating cabinet (37 °C) and a 5 % CO_2 (PeCon CO_2 controller 2000, Germany) enriched atmosphere was added to the sterile cell chamber. This ensures optimal environmental conditions for biological specimens inside the sample chamber. The cell chamber was additionally filled with Dulbecco's Modified Eagle Medium (DMEM, with phenol red indicator, else without additives and antibiotics), which was subsequently examined for possible contaminations. During the test period, two medium changes and tube changes were carried out (day 5 and 11) as in an experiment with a biological specimen.

3 Results

The sterility as well as the functionality of the realised bioreactor stimulation system could be successfully confirmed with the help of the conducted function test. After the test, the medium showed no turbidity or contamination (Fig. 4). The phenol red indicator had a constant colour throughout the test procedure and indicated a typical colouration for a slightly basic medium of pH7.4 (Fig. 2), which is due to the addition of CO_2. A constant indicator colour is a hint of a tight system and well-regulated CO_2 supply.

Furthermore, additional biocompatibility tests were carried out with the components that are in direct contact with cells. These tests also showed a good cell compatibility.

The compression force of the dummy sample was constantly monitored by the force sensor (Fig. 4) and shows a continuous decrease. This is due to the memory effect of the used foam specimen. The foam adapts its shape to the compression and has only a low restoring force. In addition, the temperature at the cell chamber was monitored. The temperature measurements also detected the opening of the heating cabinet.

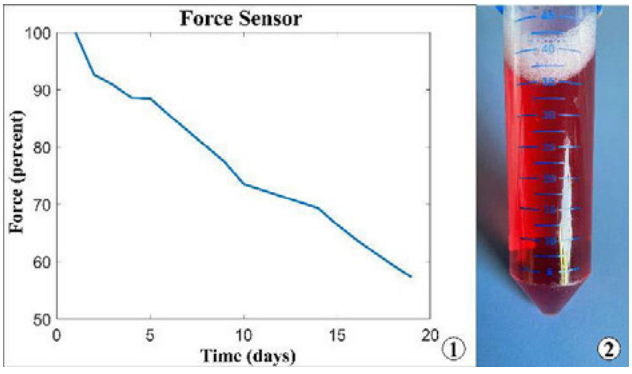

Figure 4: 1) Mean compressive force in percent during the 18-day trial run (100 percent corresponds to the start value). 2) The medium taken directly after the trial run shows no contamination.

4 Discussion

A bioreactor system is a key element in the research of three-dimensional tissues for future clinical use. A compression

bioreactor was developed to investigate the influences of long-term compression cycles on bone cell cultures and their cell formation.

By using a real-time capable controller, the bioreactor system enables fully automated tissue cultivation and cyclic stimulation of three-dimensional cell cultures in 24/7 long-term operation. The use of a fully automated bioreactor enables a consistent stimulation of the specimen and subsequent storage of the configuration and measurement data on a USB flash drive for documentation purposes. The monitoring and configuration of the experiments can be done directly at the user interface of the real-time controller, so that no additional computer is necessary, and the bioreactor can act as a stand-alone device.

The temperature of the cell chamber and the loading force on the dummy sample were recorded via control unit during the trial run. This should allow material changes in the tissue to be detected during testing. During the trial run, a change in the specimen material could be registered. However, this is not comparable with biological tissue. Therefore, this effect needs to be verified with biological specimens.

The bioreactor operates in a heating cabinet with a peristaltic pump for medium exchange and receives additionally a CO_2 supply. This ensures optimal environmental conditions for the specimens. The cell chamber also fulfils the essential boundary conditions of sterility and biocompatibility. Furthermore, the medium and the tubes can be changed during the test without the risk of contamination. In general, the handling and assembly of the bioreactor is very simple to minimise possible risks of contamination.

The successful technical implementation confirmed the compression device for the cultivation of three-dimensional cell constructs under cyclic mechanical compression loading. The next crucial step after the technical tests is to conduct an experimental study with three-dimensional cell cultures. Due to the modular and flexible design, the system can be adapted for the cultivation of different 3D cell cultures as well as for a stimulation by stretching in the future.

Author Statement

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