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SHORT COMMUNICATION

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A NOVEL BIOREACTOR WITH MECHANICAL STIMULATION FOR SKELETAL TISSUE ENGINEERING

The provision of mechanical stimulation is believed to be necessary for the functional assembly of skeletal tissues, which are normally exposed to a variety of biomechanical signals in vivo. In this paper, we present a development and validation of a novel bioreactor aimed for skeletal tissue engineering that provides dynamic compression and perfusion of cultivated tissues. Dynamic compression can be applied at frequencies up to 67.5 Hz and displacements down to 5 μm thus suitable for the simulation of physiological conditions in a native cartilage tissue (0.1-1 Hz, 5-10 % strain). The bioreactor also includes a load sensor that was calibrated so to measure average loads imposed on tissue samples. Regimes of the mechanical stimulation and acquisition of load sensor outputs are directed by an automatic control system using applications developed within the LabView platform. In addition, perfusion of tissue samples at physiological velocities (10-100 μm/s) provides efficient mass transfer, as well as the possibilities to expose the cells to hydrodynamic shear and simulate the conditions in a native bone tissue. Thus, the novel bioreactor is suited for studies of the effects of different biomechanical signals on in vitro regeneration of skeletal tissues, as well as for the studies of newly formulated biomaterials and cell biomaterial interactions under in vivo-like settings.

Key words: bioreactor; dynamic compression; perfusion; cartilage; bone; tissue engineering.

Tissue engineering is a multidisciplinary field, which applies principles of biology, chemistry, engineering, and medicine in order to address the clinical problem of the tissue and organ failure. One of the approaches to tissue engineering aims at *in vitro* cultivation of functional tissue equivalents for the potential therapeutic application by the integrated use of autologous cells, biomaterial supports, and bioreactor systems [1]. This approach is based on a generally accepted hypothesis that the cells will regenerate functional extracellular matrix (ECM) *in vitro* under the conditions that resemble the native *in vivo* environment. Thus, the design of tissue engineering systems is biomimetic in nature and aims at recapitulating biological processes during a normal tissue development.

Skeletal tissues are generally load bearing tissues in the body exposed normally to a variety of biomechanical forces. Consistently, bone cells were shown to respond to different mechanical signals under *in vitro* conditions including hydrostatic pressure and hydrodynamic shear. Specifically, tissue engineered bone growth was shown to be considerably enhanced by hydrodynamic shear so that contemporary bone bioreactors are designed to direct the medium flow through the engineered tissue in order to expose all cells to shear stresses [2-7]. On the contrary, dynamic compressive stresses are believed to be essential for the assembly of a functional cartilaginous tissue. Articular cartilage is a soft, avascular tissue lining bone endings in synovial joints with main functions to transfer mechanical loads to the subchondral bone and provide joint mobility at negligible friction without wear or tear. Under normal physiological conditions, articular cartilage is exposed to compressive stresses, which can be as high as 6-18 MPa [8]. Consistently, several bioreactors with controlled dynamic loading were proposed for cartilage tissue engineering and were configured with tissue pieces immersed in medium and pressed by a plunger on top [9-15]. However, mass transfer in this bioreactor set up is limited to outer cylindrical surfaces of the tissue specimen and may restrict the size of the engineered cartilaginous tissue.

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In this paper, we present the development and validation of a novel bioreactor for skeletal tissue engineering, in which both dynamic compression and interstitial medium flow through the cultivated tissue are provided.

BIOREACTOR SYSTEM

Bioreactor description

The novel bioreactor is custom made and configured as a unit of six cultivation cartridges placed in a cartridge holder, which is subsequently secured on a metal base (Figure 1). The base can be moved vertically by a stepper motor mounted underneath. Beneath the cartridge holder, a load cell is installed in order to provide measurements of average loads put on all six cartridges. Cartridges are cylinders made of polypropylene, supplied with 2 ports for medium transport and provide space for a biomaterial or a tissue specimen 16 mm in diameter and up to 3 mm thick. The specimen is placed on a sintered glass plate while the cartridge top is covered by a diaphragm made of Bergaflex (Termoplast DOO, Belgrade, Serbia) (Figure 1a). The diaphragm can be pressed down by a micrometer screw providing the exact initial positioning in each cartridge, independently of the differrences in the specimen thickness. Each cartridge is connected to a separate recirculation loop consisting of a medium reservoir and a silicone tubing coil serving as a gas exchanger (Figure 1c). Medium recirculation is provided by a multichannel peristaltic pump at superficial velocities through the specimen in the range of 10–100 μm/s, which correspond to blood velocities in capillaries.

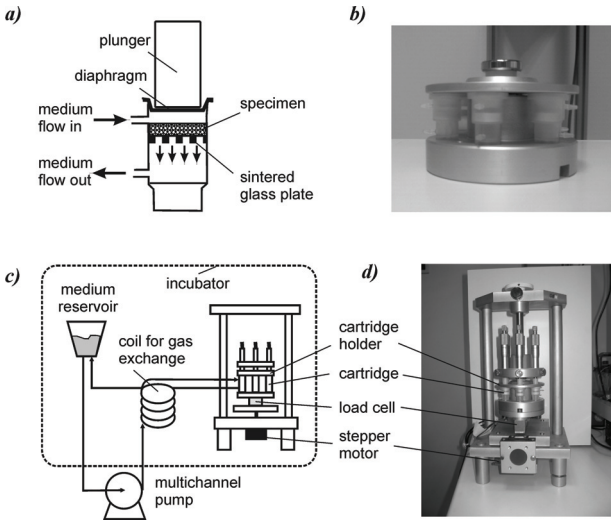

Figure 1. The novel bioreactor system; a) the cartridge with a specimen placed on a sintered glass plate; the upper specimen surface can be pressed by a diaphragm positioned by a micrometer screw; b) the cartridge holder hosting 6 cartridges; c) the recirculation loop (shown for 1 cartridge only) consisting of a gas exchanger and a medium reservoir; d) the bioreactor with the cartridge holder fit onto the base that is moved by the stepper motor mounted underneath; applied loads are measured by a load cell installed below the cartridge holder.

Control system

The bioreactor control system (Figure 2) consists of the PCI-6221 data acquisition (DAQ) card (National Instruments, TX, USA), a dedicated PC for the control of the DAQ, a stepper motor (Surestep STP--MTR-23055, AutomationDirect, GA, USA) connected to a drive (UnoLux NS d.o.o., Serbia), the load cell Scaime AL3C3SH5e (Scaime, France), and various power supplies. The dedicated PC controls two separate flows of information: one that directs movements of the bioreactor base and the other conveying outputs of the load sensor.

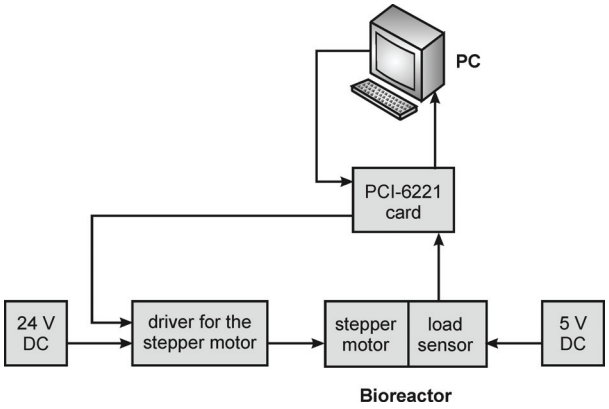

Figure 2. The bioreactor control system: the stepper motor built in the bioreactor is driven by a driver connected to a PCI-6221 DAQ card inserted in a dedicated PC; the same card also serves for the acquisition of load cell signals; the system is controlled using programs developed within the Labview platform.

The stepper motor movement is directed using an application developed within the Labview platform (National Instruments, TX, USA), which sends appropriate control signals to the motor drive. In this way, the PC application controls the speed and direction of the bioreactor base displacement. Since the steps are generated by the application, and not by the hardware, the maximal frequency of the motor movement had to be experimentally measured and amounted to 67.5 Hz. The frequency of the steps performed can be then adjusted to the desired value by the PC application. One step of the stepper motor corresponds to the motor axle shift of 1.8 degrees, hence providing precision of the vertical displacement of 5μm.

The PC is also controlling acquisition of signals from the load cell. The cell can measure weights up to 3.75 kg, with a minimal precision of 0.5 g. The output of the sensor is rated, and it depends on the power supply voltage. For the 10 V of the power supply, the output sensibility equals to 19.74 mV. The sensor output is connected to analogue inputs of the DAQ, which has a built-in amplifier, and can perform 16-bit A/D conversion in the selected input range. We have programmed the input to be in the range of ±0.2 V, so that the minimal voltage difference that can be measured is about 6.1 μV. This value is well below the load sensor sensitivity and does not decrease the precision of load measurements.

We have developed several applications within the Labview platform for the bioreactor control. The main application controls the movement of the bioreactor base and performs data acquisition from the load sensor at a given command. In this application, several parameters can be defined, such as the amplitude and the frequency of the vertical base displacement, durations of the working and pause periods, and the total number of work/pause cycles. The measurements of loads imposed on cartridges are made during the pause phase upon a command specifying the number of subsequent measurements that should be made in order to calculate the average response and minimize the influence of the noise. In addition to the main application, several other programs were developed including programs for the initial base positioning and load sensor validation.

BIOREACTOR VALIDATION

Validation of the novel bioreactor system was carried out with respect to the displacement control, calibration of the load cell outputs, and bioreactor sterility and biocompatibility.

Displacement validation

To determine the accuracy of the displacement we have performed several tests using trivial application, which moves the bioreactor base for a certain number of steps at a fixed speed. To determine the actual vertical displacement we have used a nonius with a precision of 0.1 mm. The stepper motor was commanded to perform a certain number of steps, and the actual displacement was measured. In the tests, we have varied the number of ordered steps (25, 50, 100, and 200) and in all cases we have obtained the expected values with the given precision (0.1, 0.2, 0.5, and 1.0 mm, respectively).

Calibration of the load sensor outputs

In order to determine loads imposed on cultivation cartridges, it was necessary to calibrate the outputs of the load sensor. Specific weights in the range 1 g–1 kg were put on the bioreactor base and voltage signals from the load cell were recorded. Tests were performed in several series with different cartridge loadings and repeated 10 times each. In all experiments, sensor outputs were exceptionally reproducible with standard deviations lower than 0.3 % and yielded almost ideally linear functions of the imposed weights (r^2 > 0.999, Figure 3).

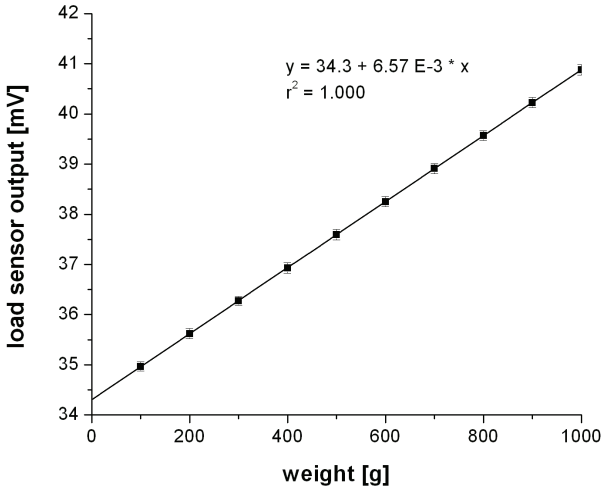

Figure 3. The output from the load sensor as a function of given loads measured in one experimental series (experimental data: symbols, and the linear regression fit : line).

Bioreactor sterility and biocompatibility

Cartridges, the cartridge holder, and silicone tubing were autoclave sterilized (30 min at 121 °C), assembled in recirculation loops under the sterile laminar hood and filled with the sterile culture medium (Dulbecco's Modified Eagle Medium (DMEM), Sigma, St. Louis, USA). The closed cartridge holder was then transferred to the bioreactor frame in the incubator providing manipulation without the risk of contamination. Cartridges were recirculated with the culture medium at a physiological rate (≈ 40 μm/s) for 2 weeks in order to verify the maintenance of the bioreactor sterility and tightness.

Biocompatibility of the bioreactor was confirmed by cell viability tests in short term static cultures of murine bone marrow stromal cells (BMSC) incubated together with sterile bioreactor parts, as well as in a 7-day bioreactor culture of bovine chondrocytes immobilized in alginate microbeads. For BMSC cultures, inbred CBA mice, 8–10 weeks old, were used as donors of bone marrow cells, which were propagated *in vitro,* as des-

cribed previously [16]. Bovine calf chondrocytes were isolated from the articular cartilage harvested from the femoropatellar grooves of 6 months old bovine calves and propagated in 2 passages according to the protocol described previously [17]. Chondrocytes were then immobilized in alginate microbeads (≈ 800 μm in diameter) at a cell concentration of 33×10^6 cell/ml by electrostatic extrusion, as described previously [18]. Three cartridges were loaded with approximately 0.5 ml microbeads each, and continuously recirculated at a flow rate of 0.3 ml/min corresponding to the superficial velocity of about 25 μm/s. Dynamic compression of the cultures was performed at the regime 1 h work period per 1 h pause with the movement frequency of 0.42 Hz and the absolute displacement of 0.4 mm. In all cultures, cell viabilities determined by trypan blue exclusion test were high (> 90 %) confirming the bioreactor biocompatibility.

CONCLUSION

Biomimetic approaches to tissue engineering provide possibilities to closely imitate the native cell microenvironment and recapitulate natural processes of the tissue development and regeneration in order to promote the functional tissue assembly *in vitro*. Here, we present a novel bioreactor with controlled dynamic compression and perfusion of cultivated tissues. Hence, it is possible to deliver biomechanical signals and establish efficient mass transport simultaneously, as well as to apply different regimes of the operation and to generate conditions resembling those in native cartilage or in native bone. The bioreactor was shown to be biocompatible and to support packed bed cultures of chondrocytes immobilized in alginate microbeads. In addition, the novel bioreactor could be used as a tool for controlled studies of biomaterials and cell-biomaterial interactions under *in vivo*-like settings.

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