Biomed Tech 2012; 57 (Suppl. 1) © 2012 by Walter de Gruyter · Berlin · Boston. DOI 10.1515/bmt-2012-4182

# Development of a cyclic multi-axial Strain Cell Culture Device

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## Abstract

Cells of many tissues are subjected to external mechanical forces. This is particularly the case for the alveolar epithelial cells of the lung, which undergoes a continuous multi-axial strain. Therefore, this study aimed at the development of a cell culture device that allows the application of multi-axial strain on lung epithelial cells in vitro at physiological and pathophysiological level in a suitable cell culture format. The mechanical part of the developed device consists of four pressure pistons each with one cell chamber to hold an elastic silicone membrane, forced by a linear motor. The motor power and direction are divided and changed by a special gear. This results in a compact design for use in standard incubators. The electronic parts, the user interface and motion controller are located outside at room temperature. The motion of the linear motor is programmable freely in a wide range. Therefore, the resulting strain vs. time can be controlled according to physiological or pathophysiological profiles. At this time an area extension of 12 to 37 % and a frequency of 12 to 40 $min^{-1}$ is used. The design of the cell chamber allows a pre-culture of adherent cells and the preparation for post-analysis.

## 1 Introduction

The successful development of a cell culture system is an interdisciplinary process. Knowledge in scientific instrumentation and biology are the basics of such a research project. Using an incremental targeted procedure for the development and testing of prototypes our project generates a useful device, which keeps open for new ideas.

### 1.1 Cellular Biomechanics

Cells of many tissues are subjected to different physical forces. If forces change the length of cells in relation to the initial length, these forces are accurately referred to strain. However, the term stretch has been used simultaneously by the researchers. *In vivo* cell strain normally occurs in all directions. This multi-axial strain is particularly applied to airways and alveolar epithelial cells in regular cycles due to the involuntary expansion and passive recoiling of the lung during respiration [1,2]. An increasing number of experimental studies demonstrated the effect of cyclic strain on the cellular physiology [3,4], cytoskeletal structure [5,6], signal transduction [7,8] and gene expression [9,10] in lung epithelial cells.
Simultaneous cyclic strain also alters the effective action of compounds such as lipopolysaccharides [11] and chemotherapeutic drugs [12] in lung epithelial cells. As in biomedical research cells that are normally subjected to physical forces *in vivo* are commonly being studied in static conditions *in vitro,* the interpretation of the resulting findings is limited. In general, there is still an incomplete understanding concerning the effect of cyclic or sporadic strain on the cellular function in different types of cells.

### 1.2 Technical Requirements

As our group primarily focuses on pulmonary research, we were interested in the development of a cell culture device that applies cyclic multi-axial strain to cell monolayers cultured on flexible-bottomed culture units at *in vivo*-relevant levels. According to the airway and alveolar cyclic strain in physiological and pathophysiological conditions [2] this device should ensure a surface area extension up to about 35 % and a frequency up to 40 $min^{-1}$. For studying time-dependencies, this cell culture device should allow independent but synchronic experiments in individual chambers of an appropriate cell culture format (about 10 $cm^2$ area of the cell layer). Moreover, the individual cell chambers should be easy to handle in the laminar flow box and cell culture incubator in order to work sterile and fast. The design of each cell chamber should allow the preculture of adherent cells and preparation for post-analysis. Further requirements were a drive mechanism based on electricity but not compressed air and the long-term resistant of the entire unit to the air conditions in the incubator (37 °C, humidified 10 % $CO_2$ atmosphere).

## 2 Methods

The centre of medical basic research has a precision mechanical workshop for prototyping at its disposal. There is also knowledge in programming of microcontrollers for the device firmware and LabVIEW for the generation of the strain vs. time profiles. The biological evidence of the mechanical effects can be shown by cell-based methods.

## 2.1 Manufacturing Methods

The CAD-Software Solid Edge (Siemens PLM, Köln, Germany) was used to design and simulate the mechanical construction. The electromechanical and electro-pneumatically components were selected in a reliable quality to meet the requirements of the continuous operation. The electrical drive consists of two components, the linear motor LM 1247-120 and the dedicated motion controller MCLM 3006 (both were from Faulhaber Minimotor SA, Croglio, Switzerland; http://www.faulhaber.com/). The motion controller is programmable by a special assembler code. The software development tool Motion Manager (Dr. Fritz Faulhaber GmbH, Schönaich, Germany) helped to generate the time critical programming and to optimize the system configuration. The graphical system design software LabVIEW (National Instruments, Austin, Texas, USA) was used to create the motion profiles and to acquire process data for the quality control.

## 2.2 Cell-based Methods

The adherent human lung epithelial cell line A549 (American Tissue Culture Collection, Manassas, VA, USA) was used to check the functionality of the *Strain Cell Culture Device*. A549 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 5 % fetal calf serum, 100 units/mL penicillin and 100 µg/mL streptomycin (all compounds were from Life Technologies, Darmstadt, Germany) at 37 °C in a 95 % humidified 10 % $CO_2$ atmosphere. The Flexcell Stage Flexer silicone membrane (Dunn Labortechnik GmbH, Asbach, Germany) was placed in the strain chamber, disinfected with 70 % ethanol, washed with phosphate-buffered saline and then coated with calf serum overnight. A549 cells ($5\times10^5$ per chamber) were seeded on the coated silicone membrane. After two days of cell growth until a confluent monolayer, A549 cells were strained at a cycle frequency of 12 $min^{-1}$ up to 24 hrs (average 10 % cell extension in length and 22 % in surface area). The cells were always covered by the same volume of medium (4 mL) to keep the simultaneously generated shear stress at constant level. After application of the strain the integrity of the cell monolayer was checked by inverted light microscopy (ECLIPSE TS100; Nikon GmbH, Düsseldorf, Germany). Actin cytoskeleton was visualized with Alexa Fluor®568 phalloidin (Life Technologies) according to manufacturer's instruction. Immunoblot detection of the mitogen-activated protein kinase (MAPK) p42/44 and the protein kinase B (PKB/Akt) in their active (phosphorylated) per inactive form was performed with antibodies from New England Biolabs GmbH (Frankfurt (Main), Germany) as described [13]. Statistics were performed by use of the SigmaPlot software 10.0 (Systat Software Inc., San Jose, CA, USA).

# 3 Results

The basic design of the cell culture device is shown in image 1. It consists of an active part located in the incubator and the controller located outside. The motion profile is saved in the EEPROM of the motion controller. Therefore, a computer is only necessary if the profile has to be changed. This is an easy and safe solution for the daily use in the laboratory.

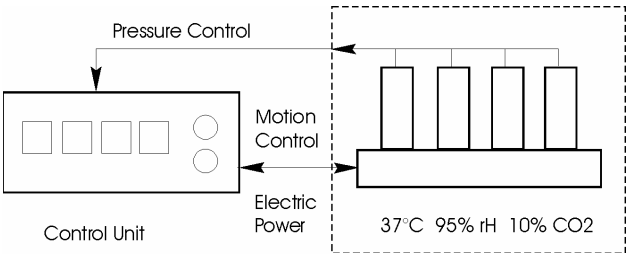


Image 1: Basic Design

## 3.1 Software

The software consists of two parts, the configuration and the control. The configuration part reflects the sensors, actors and I/O lines. The control part has to manage the time critical motion and pressure. For further information see flowchart in image 2.

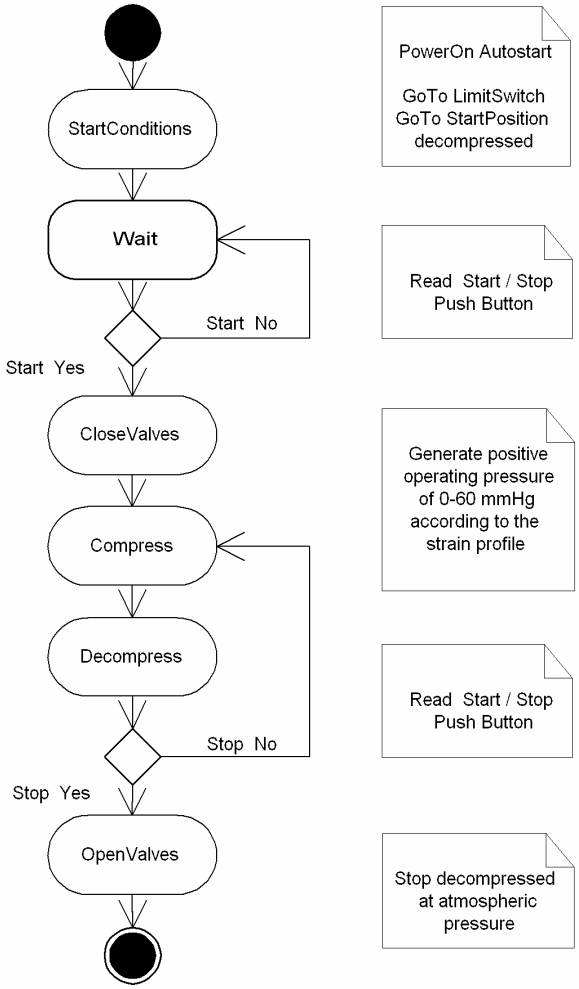


Image 2: Flowchart for motion and pressure control

The Faulhaber motion controller has a specific programming language, a mix of assembler code and elements of BASIC. The advantage is the small size of the control code segment of some 100 bytes. This makes it possible to update the complete segment in case of changing the motion profile. The connection to the host computer can be established by a serial interface cable or wireless using a Bluetooth dongle via the RS232 port.

## 3.2 Hardware

Image 3 schematically shows the connections between the main components. Motion controller and linear motor form a closed control loop to achieve a linear resolution of 7.5 µm and generate a force of up to 10 N.

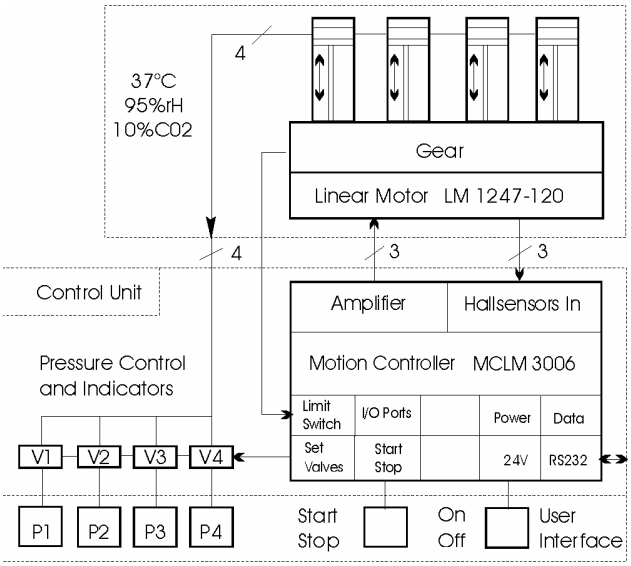


Image 3: Hardware overview

### 3.2.1 Mechanical Part

The main parts of the mechanics are four pressure pistons, driven by one linear motor. The pressure pistons are rigidly coupled to the linear drive using a gear containing toothed belts and a rack (Image 4).

Image 4: Device w/o housing, view to motor and gear

The result is an exact synchronous behaviour of all pistons and, consequently, a synchronous compression. The generated over pressure, up to 60mmHg, is transmitted by air tubes to four indicators at the user interface. In contrast to the commercially available *Flexcell Strain Unit*, which provides uniform radial and circumferential strain to a membrane surface by applying vacuum pressure to a membrane array (Flexcell International, McKeesport, PA, USA; see http://www.flexcellint.com/), our *Strain Cell Culture Device* uses a mechanical not a pneumatically coupling. In addition, the design of the cell chambers allows a pre-culture and a post analysis in a fast and efficient workflow. Image 5 shows the *Strain Cell Culture Device* inside the incubator using two cell chambers.

The multi-axial strain of cells in both models causes a cellular deformation in all directions but the intensity of strain depends on the radial position of the cell. Although this heterogeneous distribution of the strain might be of disadvantage in observing significant cellular effects for all cells together, it well reflects the *in vivo* situation [2].

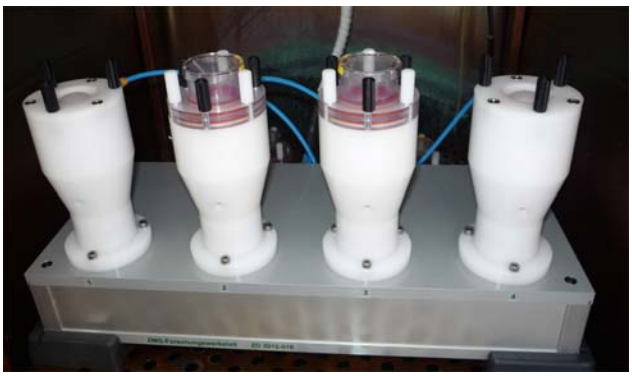

Image 5: *Strain Cell Culture Device* inside the incubator

### 3.2.1 Control Unit

The unit includes all parts for the process control and the user interface. Four pressure indicators (SUNX DP-100) and two push buttons provide the user with the relevant information, (Image 6). To get the same initial conditions in all cylinders, four electronic valves can be set by the software to decompress the pneumatic system. The serial interface RS232 is used to get online access to the motion controller by the host computer. The hosted mode makes it possible to acquire dynamic characteristics of the motor and to change the control software.


Image 6: Control Unit

### 3.3 Cell-based Application

The functionality and reliability of the *Strain Cell Culture Device* (Images 4–6) was tested by use of A549 alveolar epithelial cells, which form a homogenous cell monolayer on elastic silicone membranes. The cyclic deformation of elastic membranes leads to chronic elongation and relaxation of cells growing attached on the surface of the membrane thereby causing defined cellular changes. In this regard, short-term activation of p42/p44-MAPK with subsequent deactivation [7] as well as long-term reorganization of the actin cytoskeleton [6] has been shown for alveolar epithelial cells. These changes could also been demonstrated for A549 cells subjected to cyclic multi-axial strain in our *Strain Cell Culture Device* (Image 7A, B). Moreover, we observed a short-term activation of the protein kinase PKB/Akt in A549 cells (Image 7B).

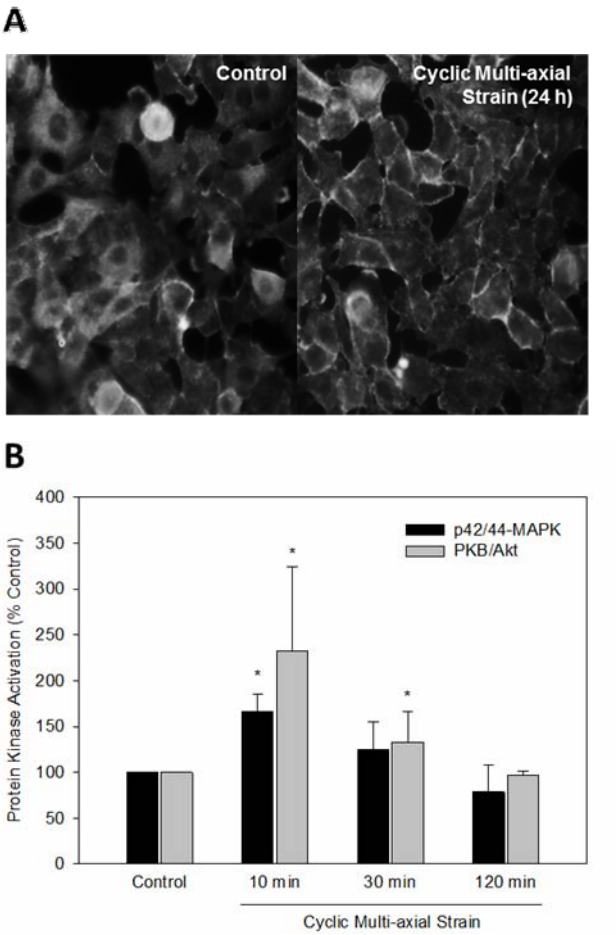


Image 7: Actin cytoskeleton staining of A549 cells subjected to long-term strain and unstrained controls (A). Strain-induced activation of protein kinases in A549 cells depending on time (*P<0.05 vs. controls, n=3) (B).

Although it has to keep in mind that the medium covering the A549 cells additionally mediates turbulent shear stress on the apical side of cells, our initial findings suggest the future application of our *Strain Cell Culture Device*. This might also include studies on other types of cells, which are physiologically influenced by cyclic (or sporadic) multi-axial strain. In this regard, our device might be suitable for studies using adherent cells such as endothelial cells, smooth muscles cells and fibroblasts as well as semi-adherent cells such as monocytes.

## 4 Conclusion

The development of the new *Strain Cell Culture Device* is a result of the closed cooperation between users in the clinical basic research and constructors in the biomedical engineering field. The device is a prototype based on the knowledge of constructing similar systems to apply mechanical forces to cells.

## 5 Acknowledgement

The authors thank Dieter Barowsky for his technical assistance. This project was supported by grants of Deutsche Forschungsgemeinschaft (BA 2077/4-1) and Wilhelm Roux program at the Medical Faculty Halle (Saale) (FKZ 13/23, FKZ 21/43).

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