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# New bioreactor vessel for tissue engineering of human nasal septal chondrocytes

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**Abstract:** Cultivation of human nasal septal chondrocytes in a self-established automated bioreactor system with a new designed reactor glass vessel and the results of a computational fluid dynamics model are presented. The first results show the effect of a homogeneous fluidic condition of the continuous medium flow and the resulting stresses on the scaffolds' surface and their influence on the migration of the cells into the scaffold matrix under these conditions. For this purpose computational models, generated with the computational fluid dynamics software STAR-CCM+, and the results of alcian blue staining for newly synthesized sulphated glycosaminoglycans have been compared during cultivation in the new and a first version of the glass reactor vessel with inhomogeneous fluidic conditions, with the same automated bioreactor system and under similar cultivation conditions.

**Keywords:** automated bioreactor system; cell ingrowth; computational fluid dynamics; cultivation vessel; dynamic cultivation; facial cartilage; scaffold colonization.

## 1 Introduction

Defects of septal or auricular facial cartilage are congenital or are caused by trauma or cancer. For reconstruction, biocompatible artificial implants or autologous donor tissue, harvested from the ribs or concha are used [1]. The advantage of the former method is e.g. limitless supply of implants [2], of the latter the lack of rejection reaction. Both have certain disadvantages such as extrusion, infection or donor site morbidity [3].

To solve those problems tissue engineering is a promising approach. In this approach, the patient's own cells are seeded on a so-called scaffold, a three-dimensional porous structure made of a biocompatible material, and so cartilage can grow in a predefined shape [4]. To offer best possible conditions to the cells, different kinds of bioreactors had been established over the past years [5, 6]. Depending on the type of cultivated cells or intended type of cartilage different varieties of stresses and nutrition supply are necessary to lead to the desired results. To get an idea of these stresses the cells are exposed to or of the fluid dynamics in the bioreactor, computational modelling e.g. with CFD simulations can be used [7].

The outcome of the CFD modelling for the first version of the glass vessel of the established bioreactor system is presented in Figure 1. It shows inhomogeneous fluidic conditions and the resulting inhomogeneous wall shear stresses on the scaffolds' surface. A detailed description of the established bioreactor system with the first version of the glass vessel and first results of cultivations can be found in [8].

## 2 Material and methods

### 2.1 Automated bioreactor system

The bioreactor system presented here (Figure 2) consists of several components which are necessary to realize ideal cultivation conditions for the cells and the automatic sequence control of the whole system controlled by a self-established operating program via LabVIEW 2014 (National Instruments, TX, USA).

This system offers the opportunity to control and regulate most of the environmental conditions like gassing, medium flow and medium exchange, heating of the bioreactor vessel and the non-invasive optical measurement of $O_2$ and pH in the medium.

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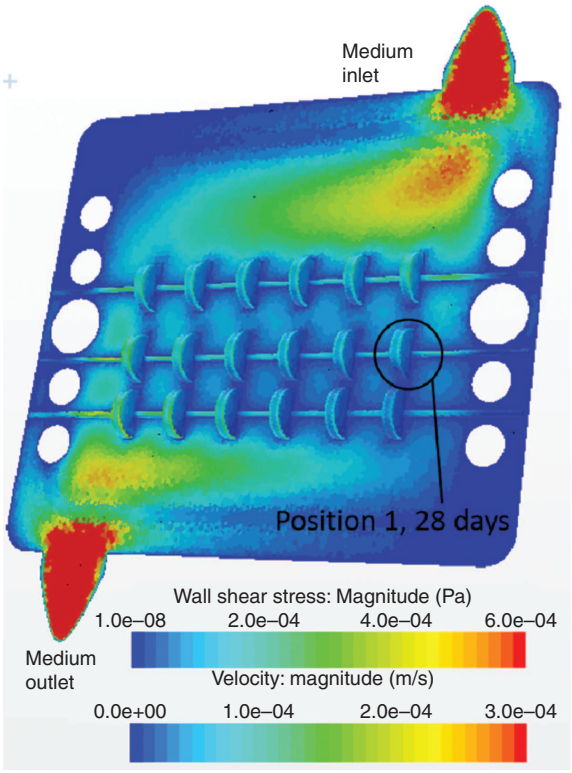


**Figure 1:** CFD model of the former glass reactor vessel with the speared scaffolds on the wires during continuous medium flow of 2 ml/min during cultivation. It shows the velocity magnitude of the media on its way from the inlet to the outlet and the wall shear stress on scaffolds' surface.

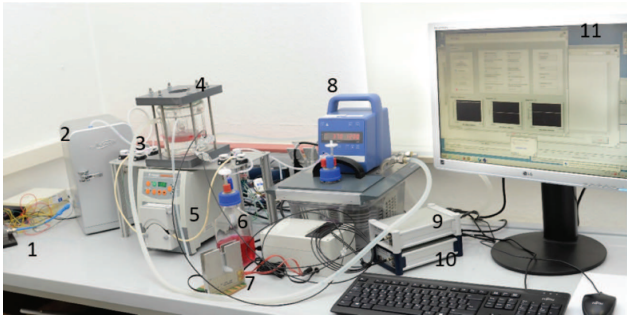


**Figure 2:** Photograph of the complete bioreactor system during a cultivation of populated scaffolds. Following components are contained: 1. Mass flow controller for gassing, 2. Refrigerator with fresh medium reservoir, 3. 6 Pinch valves for the media flow regulation, 4. Glass vessel bioreactor, 5. Peristaltic pump, 6. Bottle for medium waste, 7. Heating unit for exhaust gas filter, 8. Water bath with glass bottle for medium preheating, 9. & 10. Fibre optic transmitters for $O_2$ and pH measurement, 11. Control PC.

For that purpose, the glass vessel has a double jacket for the heating and contains inlets and outlets for the gassing and medium flow. Inside this vessel are two removable flow conducting elements, one after the medium inlet and one in front of the medium outlet, which lead to a homogeneous medium flow inside the vessel. Two mobile displacement devices for the medium can also be built in on demand. The dimension of the glass vessel's cultivation chamber is 80 mm in diameter and could be implemented with an attachment for three wires or another attachment for auricular shaped scaffolds.

All further components of the system are fully described in [8].

## 2.2 Cells, scaffolds and media

Human primary nasal septal chondrocytes, obtained during routine surgeries in the Department of Otorhinolaryngology at the University Medical Center Ulm, were seeded on scaffolds made of sterile and processed porcine nasal septal cartilage, prepared at the Institute of Bioprocess Engineering of the University of Erlangen, with a diameter of 5 mm and 1 mm thickness. More detailed information about pre-treatment and preparation of the cells and scaffolds are reported in [9, 10].

The medium used here was StemMACS ChondroDiff Media human, a chondrocyte differentiation medium, from Miltenyi Biotec GmbH (Bergisch Gladbach, Germany).

## 2.3 Cell seeding

After 4 days of cultivation in monolayer culture, the cells reached subconfluence at 80–90% and a cell suspension with a concentration of $5 \times 10^6$ cells/ml was produced. Subsequently, each scaffold was seeded with about $1 \times 10^6$ cells and incubated in standard basal culture medium at 37°C with 5% $CO_2$ under humidified conditions for about 1 h to allow cell adhesion. More detailed information is presented in [9, 10].

## 2.4 Cultivation conditions

The cultivation of the seeded scaffolds was performed in the automated bioreactor system for 6 weeks. Six scaffolds were placed on each of three Kirschner wires with diameters of 0.6 mm. Every 2 weeks, one wire was removed for qualitative and quantitative analysis of the corresponding six scaffolds. The temperature was 37°C, the pre-mixed gas consisted of 5% $CO_2$, 20% $O_2$ and 75% $N_2$ and the flow rate of the media was 2 ml/min. The automated medium exchange was performed twice a week with a flow rate of 20 ml/min, exchanging 2/3 of the total volume.

### 2.5 CFD

For a first impression about the stresses on the scaffolds' surface and the fluid flow characteristic a CFD modelling was performed. This was performed with the software STAR-CCM+ version 9.06.011 from CD-adapco (Melville, NY, USA) find a correlation between the computational results and laboratory analyses. The software ran on an iCore 5 PC with 3.2 GHz and 32 GB Ram.

### 2.6 Histology and immunohistochemistry

As further qualitative analysis, histological (alcian blue) and immunohistochemical detection methods (IHC) of newly synthesized sulphated glycosaminoglycans (GAGs) such as aggrecan were performed. In addition IHC was also performed for collagen type I and II. Additional data are given in [9, 10].

## 3 Results

The CFD model in Figure 3 illustrates the velocity magnitude of the continuous medium flow during the cultivation in the reactor vessel on its way from the medium inlet to the medium outlet. The model also depicts the wall shear stress on the scaffolds' surface resulting from the continuous medium flow. Figure 4A and Figure 4B present the results of the alcian blue staining visualising sulphated

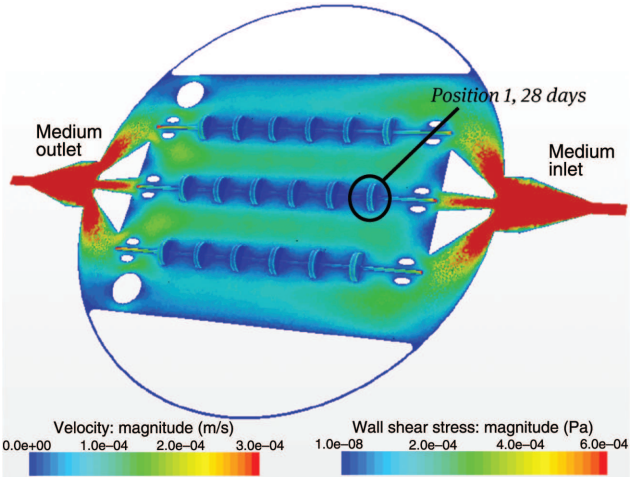


**Figure 3:** CFD model of the new glass reactor vessel with the speared scaffolds on the wires during continuous medium flow of 2 ml/min during cultivation. It shows the velocity magnitude of the media on its way from the inlet to the outlet and the wall shear stress on scaffolds' surface.

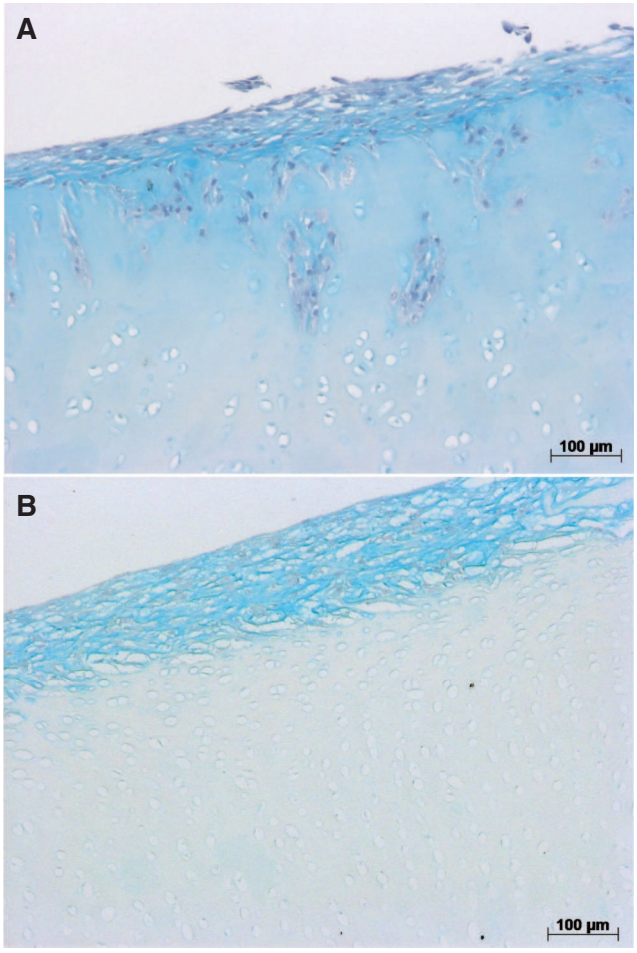


**Figure 4:** (A) Results of alcian blue staining for newly synthesized sulphated GAGs after 28 days of cultivation at position 1 in the new reactor vessel. (B) Results of alcian blue staining for newly synthesized sulphated GAGs after 28 days of cultivation at position 1 in the former reactor vessel. The comparison reflects that in (A) the migration of the chondrocytes into the scaffold matrix is deeper and more GAG was produced in the inner region of the scaffold.

GAGs. By the blue staining of the newly synthesized sulphated GAGs the migration of the chondrocytes into the matrix of the scaffolds is clearly representable. Figure 4 demonstrates the alcian blue staining for scaffolds at position 1 after 28 days of cultivation in the (A) newly established reactor vessel in contrast to (B) in the former vessel. These two scaffolds have been chosen because of their similar position in the two different reactor vessels to show a potential difference in cell migration. In the new vessel the cells migrate into the matrix and generate a superficial cell layer on the scaffold surface which demonstrates the presence of newly synthesized GAGs. Cells in the former vessel are able to generate the superficial layer and produce new GAGs as well, but show no migration behaviour. These differences in cell migration might result from the

different flow conditions and consequential stresses on the scaffolds' surface.

## 4 Discussion and conclusion

The comparison of the computational results of the former (Figure 1) and new (Figure 3) bioreactor glass vessel proves clearly that the medium flow during continuous flow is more homogeneous in the new vessel which might lead to better results concerning the intended migration of chondrocytes into the scaffold matrices. This homogeneous flow also leads to a better nutrient supply and waste removal from the cells, also in deeper structures of the scaffold matrix. The comparison of Figure 4A to Figure 4B reveals that the cells on the scaffold in the new vessel, with optimized geometry, show better migration behaviour than the one in the former vessel on comparable positions in the bioreactor after the same duration of cultivation. The difference in migration behaviour of the chondrocytes could also be caused by the surface structure of the scaffolds.

The conclusion is that the new geometry of the reactor vessel leads to better results concerning cell migration into the scaffold matrix in all the scaffolds regardless of their position in the vessel. This might result from the nearly homogeneous medium flow characteristics and the consequential similar stresses like pressure and wall shear stress on the scaffolds.

These first results will be evaluated in more detail by additional cultivations to obtain more detailed information on cell behaviour under the above mentioned cultivation conditions.

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