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Investigation of bone marrow stem cells in the bone marrow niche in an *in vitro* system

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Introduction

In our study we aim to develop an *in vitro* culture system to mimic the human bone marrow stem cell niche in an artificial perfusion bioreactor environment to culture human adult stem cells. State of the art human bone marrow stem cell research shows that even the smallest changes in the physical, thermo dynamical or biochemical environment induce a differentiation of human bone marrow stem cells into other cell lines such as e.g. fibroblasts or osteoblasts. This is undesirable for bone marrow stem cell maintenance *in vitro*. With this new bioreactor system we intend to keep the viable human bone marrow stem cells in a quiescent state, as they are in the human long bone niche. This new system will give a new insight into the bone marrow stem cell niche, concerning the cell development for maintenance as well as for proliferation. Additionally we aim to investigate how the bone marrow stem cells are initially activated and at which point in the cell development the differentiation process outweighs the proliferation process. Up until now, a detailed characterisation of the mesenchymal stem cell is still missing and this would reduce the confusion which arises from the different culture methods in monolayer. Knowledge of the mesenchymal stem cell niche will greatly improve the methodologies used later in future bone marrow stem cell research. This project will lead to a valuable and reproducible source of stem cells for musculoskeletal repair. The technology can be used in other fields where stem cells are required, such as leukaemia, heart disease, vascular injury and neuronal injury.

Methods

In the literature [1],[2] there are several descriptions that shear stress is undesirable for the maintenance of mesenchymal stem cells *in vitro* because they start to differentiate into other cell lines e.g. fibroblasts or osteoblasts due to this mechanical loading. These results encourage us to start first with a simulation model to verify a new bioreactor design. A new 3-D perfusion bioreactor was designed with a computer aided design (CAD) program and the laminar Navier-Stokes fluid dynamic flow inside of the bioreactor for very small Reynolds numbers simulated with the multiphysics program (COMSOL Multiphysics®) with their computational fluid dynamic module (CFD). All constraints and boundary conditions for the simulation were set as they are in the incubator so that the simulation model represents the reality of the environment in the best way (e.g. temperature, density, velocity). After this verification phase all parts were manufactured according the CFD simulation results as they were designed with the 3-D CAD program. Afterwards a first perfusion bioreactor experiment was done with a sterilized PU-scaffold tube and a sheep bone marrow sample of 20 ml with approximately 20 mil bone marrow stem cells.

Results

Figure 1 shows the fluid flow magnitude at a very low fluid flow rate inside of the bioreactor. In comparison to figure 1, figure 2 shows a pronounced high fluid flow magnitude in the center of the bioreactor at a high fluid flow rate. In figure 3 are the constant and unidirectional streamlines inside of the bioreactor at a low laminar fluid flow rate. In contrast to figure 3, figure 4 shows the development of a turbulent flow rate streamlines inside of the bioreactor with shear stress in the center. In figure 5 we see a bright field microscope picture of a Live/Dead staining of the PU scaffold sample with the cells attached to the sample from the perfusion bioreactor experiment. In green are Live cells whereas in red are dead cells. In figure 6 we see as comparison the control PU scaffold with more red cells on the sample surface. The figure 5 is the achievement of 3 days initial seeding in culture media afterwards with three days of perfusion culture inside of the bioreactor.

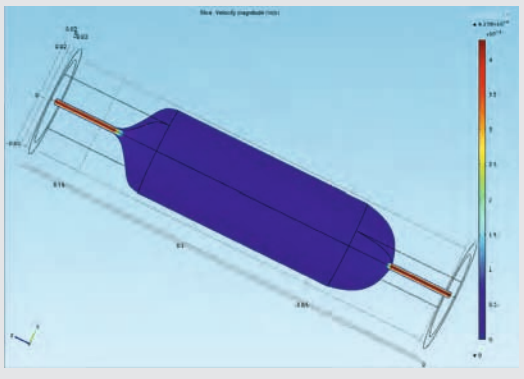

Figure 1. Fluid flow magnitude at low flow rate inside bioreactor.

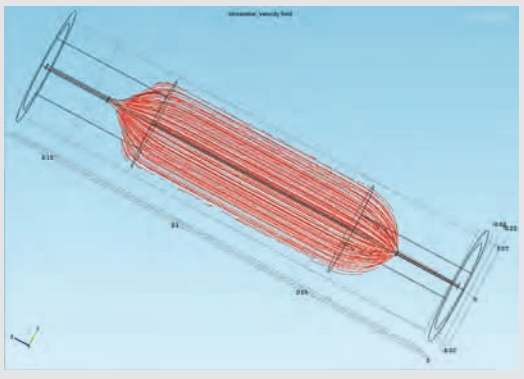

Figure 3. Fluid flow streamline at low flow rate inside bioreactor.

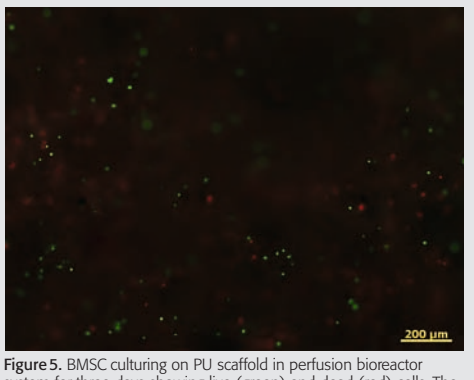

Figure 5. BMSC culturing on PU scaffold in perfusion bioreactor system for three days showing live (green) and dead (red) cells. The number of live (green) cells is greater than seen in figure 6.

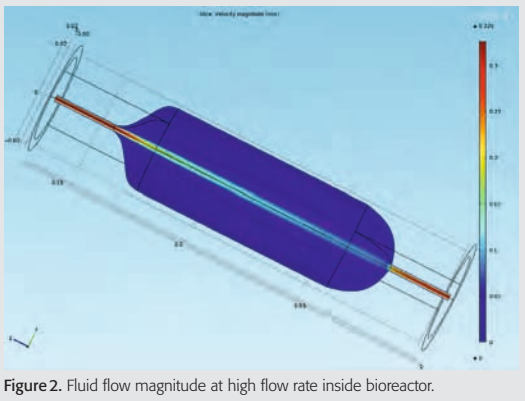

Figure 2. Fluid flow magnitude at high flow rate inside bioreactor.

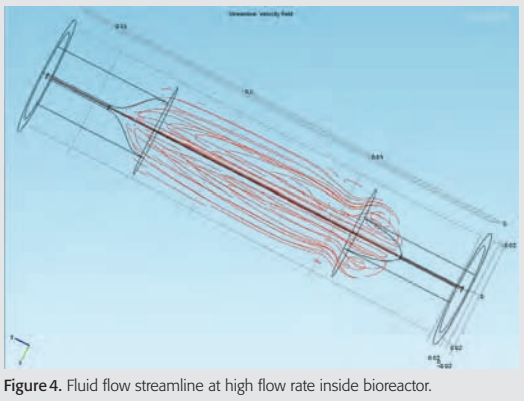

Figure 4. Fluid flow streamline at high flow rate inside bioreactor.

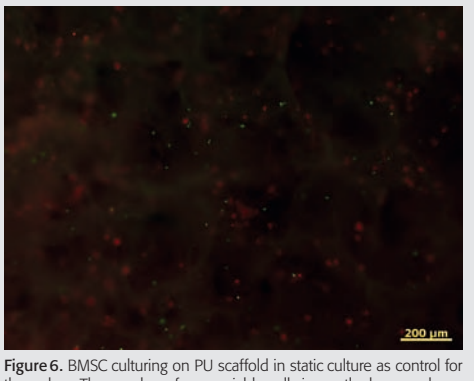

Figure 6. BMSC culturing on PU scaffold in static culture as control for three days. The number of green viable cells is greatly decreased.

Conclusion

First results show promising results in determining the bone marrow niche environment *in vitro*. They show that the thermodynamic microenvironment is very important for mesenchymal stem cell maintenance in the bone marrow niche. Even smallest changes in this microenvironment will result in differentiation of the mesenchymal stem cells into other cell lines (e.g. fibroblasts or chondrocytes). It can also result in a cell movement of up to 0.1 mm/h in the case that the local microenvironment is not sufficient for maintenance or proliferation. In the next steps we will address new questions such as how long can the mesenchymal stem cells remain quiescent in the maintenance status. Alternatively the effect of pre culture of mesenchymal stem cells onto a scaffold for critical size bone defect implants in the new perfusion bioreactor beneficial for later implantation into an animal model can be investigated. Finally it can result in a perfusion bioreactor system for the clinical application worldwide.

References
[1] M. Devarapalli et al., Biotechnol Bioeng. 103(5) 2009, 1003–1015
[2] F. Boschetti et al., Journal of Biomechanics 39 2006, 418–425

Acknowledgments
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