

Three-dimensional laminar flow dynamic culture of human adipose stem cells stimulates cell differentiation and extracellular matrix deposition

B. Weyand¹, C. Kasper², M. Israelowitz³, C. Gilles³, H.P. von Schroeder^{3,4}, K. Reimers¹, P. M. Vogt¹

¹Department of Plastic, Hand and Reconstructive Surgery, Hannover Medical School, Hannover, Germany, ²Institute of Technical Chemistry, Leibniz University, Hannover, Germany, ³Biomimetics Technologies Inc, Toronto, Canada, ⁴Department of Surgery, University Hand Program and Bone Lab, University of Toronto, Toronto, Canada

INTRODUCTION: Bioreactors for tissue engineering improve cell mass transport within three-dimensional tissue-engineered constructs. Furthermore, externally applied mechanical stress such as flow or pressure may support cell-matrix differentiation. We have recently designed and developed a novel model of a laminar flow reactor based on computer based simulations^{1,2}. The purpose of this study was to evaluate the effect of controlled three-dimensional laminar flow on cellular performance during cultivation of cell-matrix constructs in a laminar flow reactor.

METHODS: Primary human adipose mesenchymal stem (haMSCs) cells were obtained from abdominal fat tissue of patients undergoing abdominoplasty by collagenase digestion. Cells were expanded and cultured in stem cell media without addition of differentiating agents such as dexamethasone or specific growth factors. Stem cells were characterized by flow cytometry and by their capacity to differentiate into the adipogenic, osteogenic and chondrogenic pathway. Stem cell-seeded macroporous ceramic scaffolds were cultured inside a laminar flow bioreactor for up to 3 months and analyzed by surface microscopy, grinding sectioning, extracellular matrix staining and electronmicroscopy.

RESULTS: Cell-seeded matrices cultured under laminar flow demonstrated uniform cell distribution and tissue growth inside the porous structure. Scaffold analysis revealed deposition of extracellular matrix components within the scaffold. Particles released into the media during dynamic culture of cell-seeded but not of empty scaffolds stained positive for minerals, calcium derivatives, proteoglycans and collagen. Infrared spectroscopy was positive for calcium and phosphate. XR-diffraction analysis of released particles showed hydroxyapatite as well as organic components.

DISCUSSION & CONCLUSIONS:

Controlled laminar shear forces promote osteogenic differentiation with matrix deposition of adipose mesenchymal stem cells grown on a porous ceramic matrix without addition of any growth factor or hormonal differentiating stimuli such as dexamethasone in the culture medium. We propose that laminar flow is a suitable mechanical environment for differentiating osteogenic cells and that the laminar flow reactor has potential for growing bone substitutes *in vitro* for bone tissue engineering.

REFERENCES: ¹ Israelowitz *et al*, 2007, United States Patent Application 60838494/11, 895645

² Israelowitz *et al*, 2008, European Union Patent 08011144.6/EP 08011144

ACKNOWLEDGEMENTS: This work was supported by an internal start-up grant from Hannover Medical School.