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Development of a new *in vitro* tenogenic differentiation model

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Introduction: Multipotent mesenchymal stromal cells (MSC) are being used with favorable success for tendon regeneration in large animal models as well as in equine patients. Tenogenic differentiation and subsequent matrix synthesis by the MSC was hypothesized to be one of the mechanisms of MSC-supported tendon healing. However, the tenogenic differentiation pathway is not yet completely understood. It is acknowledged that not only growth and transcription factors, but also the extracellular matrix or scaffold composition and mechanical stimulation play a crucial role. Therefore, our aim was to combine natural tendon scaffolds and mechanical stimulation in an *in vitro* bioreactor model as a basis for the investigation of MSC tenogenic differentiation.

Materials and Methods: To obtain scaffolds with natural matrix proteins in parallel alignment, large equine tendons were decellularized as described previously (Burk et al., 2013). 2 mm thick scaffolds were cut from the decellularized tendons and subjected to mechanical testing. Based on the results of the mechanical scaffold assessment, a cyclic strain bioreactor prototype was designed. The prototype was tested using equine adipose derived MSC which were cultured on the tendon scaffolds and subjected to cyclic strain stimulation (2 % strain; 1 Hz; 15 min stimulation- 15 min relaxation- 30 min stimulation). Samples were assessed 24 h later by LIVE/DEAD® staining, histology and real-time RT-PCR.

Results: Mechanical testing of the scaffolds revealed that forces approximating 500 N are required for stretching of the tendon scaffolds. The bioreactor was conceptualized and built based on the design of previously described cyclic strain devices (Diederichs et al., 2010). Customizing the design to the requirements of native tendon scaffold stretching most importantly included a new clamp type to fix the scaffolds, a medium bath and a 1 kN motor with an adapted cooling system. MSC culture in the bioreactor system was successful. Cells remained viable and attached to the scaffold during the mechanical stimulation. RT-PCR allowed to assess the regulation of tendon marker genes and revealed an upregulation of collagen 3A1 following the stimulation regime used for the prototype testing.

Discussion and Conclusions: The bioreactor prototype is functional and useful for further investigation of tenogenic differentiation.

Biography

Cornelia Kasper completed her PhD in 1998 from Leibniz University of Hannover (Germany) and her habilitation in 2007 at the Institute for Technical Chemistry at the Leibniz University of Hannover. She was appointed as full University Professor for "Biopharmaceutical Production and Technology" at University of Natural Resources and Life Science (BOKU) in Vienna (Austria) at the Department of Biotechnology. She has published more than 80 papers in reputed journals and several book chapters and is editor within the series "Advances in Biochemical Engineering and Biotechnology" (Springer) of several volumes covering actual areas in Tissue Engineering and Stem Cell Research. She is also reviewer for many distinguished journal within the field of biotechnology/bioprocess and bioreactor design and enableling technolgies for stem cell cultivation.

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