TISSUE AND ORGAN MECHANOBIOLOGY

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Research Profile

The Tissue & Organ Mechanobiology (TOM) Group of the Institute for Surgical Technology and Biomechanics (ISTB), University of Bern, conducts translational research in the intersection of tissue engineering, biology and applied clinical research. The group's primary aim is to understand the cellular response onto biomechanical stimuli and how cellular communities are affected *in situ* using 3D tissue and organ culture models. Their research can be divided into two main foci: On the one hand the group investigates causes of low back pain due to intervertebral disc (IVD) degeneration and on the other hand the group focuses on the human knee where they aim to identify cell-based solutions for the non-healing or delayed ruptures of the anterior cruciate ligament (ACL). The common focus of the TOM group is to advance *in vitro* organ culture models, which match closely the human situation and where regenerative therapy strategies, such as novel biomaterials and cells, can be tested in a most authentic *in vitro* set-up.

Low Back Pain and Intervertebral Disc Degeneration and Regeneration

The TOM group conducts research in two main directions: i) IVD research in the area of regeneration using biomaterials and stem cells and ii) in the area of non-successful spinal fusion and possible involvement of pseudo-arthrose. For the first research area we use a combination of 3D tissue and organ culture approaches. The research of the second focus is the understanding of the balance between BMP agony and antagony. Besides the investigation of the exogenous stimulation of BMP antagonists on mesenchymal stem cells and osteoblast, the main focus is on the observation of the interaction between IVD cells and osteoblast, by performing co-cultures.

In a Gebert Rüf financed project a novel type of silk material has been successfully investigated for IVD repair. Here, the TOM group conducted research into new growth-factor-enriched silk, which has been produced from genetically transduced silk worms (*Bombyx mori*), which embed the growth factor of interest directly into the silk (Figure 2). The new "advanced" biomaterial has been tested *in vitro* on disc cells and mesenchymal stem cells but also in our 3D bovine organ culture model and the complex loading bioreactor together with a FDA-approved fibrin hydrogel. Therefore,

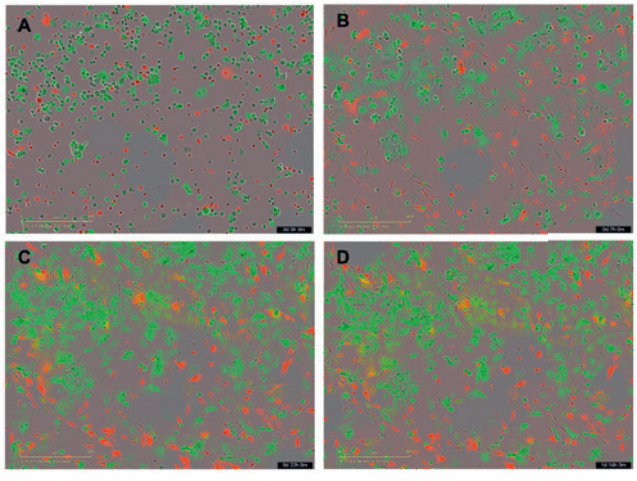

Figure 1. Time-lapse microscopy of co-cultured populations of donor-matched human primary mesenchymal stromal cells (green cytoplasmatic dye) and cartilagenous endplate cells (red membrane dye). **A**) Plating of cells (baseline) **B**) after 7h **C**) and after 22h and **D**) after 1d and 14 min of cultivation. Picture acquired with Incucyte S3 Microscope (Essen Bioscience, inc.).

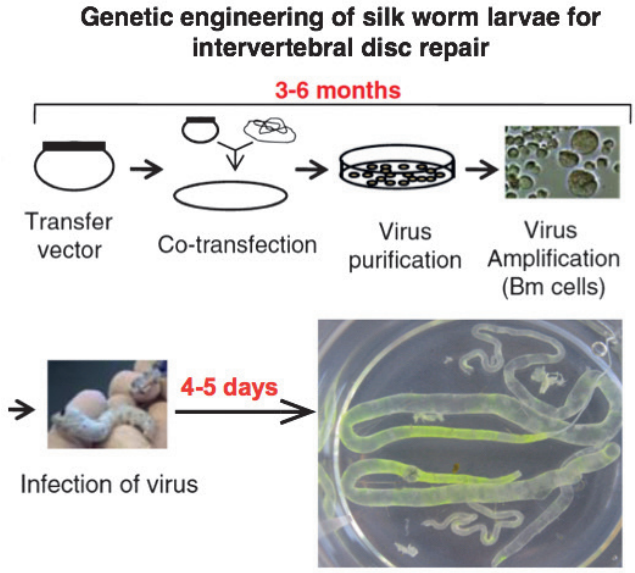

Figure 2. Genetically enriched silk fleece scaffolds were produced by transduction of *Bombyx mori* larvae with a baculovirus construct containing GDF6 or TGFβ3. Silk fleeces were produced under GMP-compliant conditions for the purpose of intervertebral disc repair.

a healthy control, an injured IVD (2 mm biopsy punch) and the repaired IVD were tested and histology was performed to visualize the injury and integration of the novel silk and fibrin hydrogel. These results were recently reported in the Journal of Orthopaedic Research.

Recently, autochthonous progenitor cells were detected in the human IVD, which could lead the path to cell therapy. Here, we concentrated on the most suitable isolation protocols to "fish" nucleus pulpous progenitor cells (NPPC) from the total population of cells in the bovine coccygeal disc. We also focused on their multipotency capacity and their application for IVD repair (Figure 3). Future research is to understand how these cells can be best isolated and whether these cells can be maintained *in vitro* to regenerate the IVD.

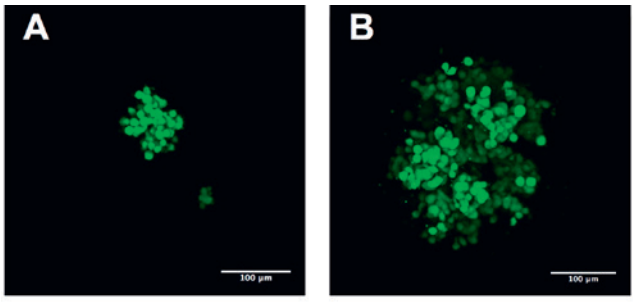

Figure 3. Confocal Laser Scanning Microscopy of **A**) nucleus pulposus progenitor cells (NPPC) and **B**) nucleus pulposus cells (NPC) after seven days of colony unit forming assay in a viscous medium. NPPC do result in more dense and spherical colonies whereas NPC form more lose and wider spread colonies. Cells were stained with a live dye in green. Scale bar = 100 µm.

The most recent branch of research in the TOM group is the investigation into non-viral gene transfer to regenerate the IVD. Here, first results were achieved to identify efficient parameters to electroporize human and bovine IVD cells and to transfer plasmid DNA to manipulate transiently the expression profile.

Biological Repair of the Ruptured Anterior Cruciate Ligament

Anterior Cruciate Ligament (ACL) injuries are very common. In Switzerland, the incidence of ruptures is estimated at 32 per 100,000 in the general population and in the sports community this rate more than doubles. Current gold standard for ACL repair is reconstruction using an autograft. However, this approach has shown some limitations. A new method has been heralded by the Knee Team at the Bern University Hospital (Insel Hospital) and the Sonnenhof clinic called Dynamic Intraligamentary Stabilization (DIS), which keeps the ACL in place in order to promote biological healing and makes use of a dynamic screw system. Here, cell-based approaches using collagen patches or application of platelet- rich plasma (PRP) are of interest. The aim of our research was to investigate the use of collagen patches, the application of PRP and platelet-rich fibrin (PRF) in combination with DIS to support regeneration of the ACL and to quantify the biological response. In a scientific excellence project (Turkey-Switzerland) 3D printed scaffolds for miniaturised ACL are currently being investigated (Figure 4). Furthermore, molecular investigations in combination with live cell imaging are ongoing to find evidence for the reduced wound healing potential of the ACL (Figure 5).

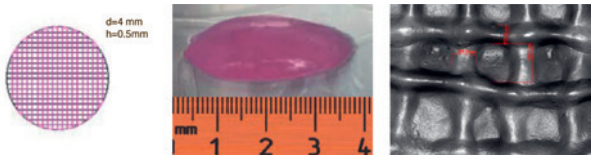

Figure 4. The computer-aided design (CAD) model that was chosen for 3D bioprinting of a gelatine and fibrin hydrogel for ACL engineering. The construct was then further characterized by the fibre and pore size under the light microscope.

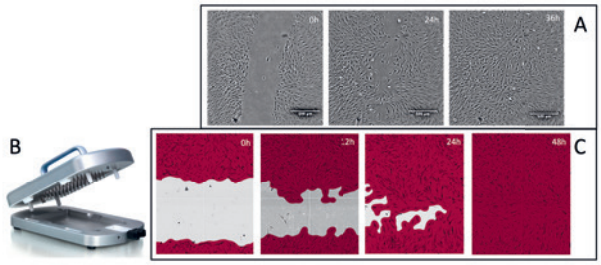

Figure 5. Wound scratch assays (WSA) using live cell imaging systems of primary ligamentocytes isolated from human anterior cruciate ligament (ACL). **A**) WSA with Nikon Biostation CT (Nikon) **B**) Woundscratcher tool™ by Essen Bioscience and **C**) WSA using Incucyte S3 Microscope (Essen Bioscience, inc.).

Publications

1. Tekari A, May RD, Frauchiger DA, Chan SCW, Benneker LM, Gantenbein B (2017) The BMP2 variant L51P restores the osteogenic differentiation of human mesenchymal stromal cells in the presence of intervertebral disc cells. Eur Cell Mater 33: 197-210 doi: 10.22203/eCM.v033a15
2. Frauchiger DA, Tekari A, Wöltje M, Fortunato G, Benneker LM, Gantenbein B (2017) A review of the application of reinforced hydrogels and silk as biomaterials for intervertebral disc repair. Eur Cell Mater 34: 271-290 doi: 10.22203/eCM.v034a17
3. Frauchiger DA, Heeb SR, May RD, Wöltje M, Benneker LM, Gantenbein B (2017) Differentiation of MSC and annulus fibrosus cells on genetically-engineered silk fleece-membrane-composites enriched for GDF-6 or TGF-β3. J Orthop Res [epub ahead of print] doi: 10.1002/jor.23778
4. Krismer AM, Cabra RS, May RD, Frauchiger DA, Kohl S, Ahmad SS, Gantenbein B (2017) The biologic response of human anterior cruciate ligamentocytes on collagen-patches to platelet-rich plasma formulations with and without leucocytes. J Orthop Res [epub ahead of print] doi: 10.1002/jor.23599
5. May RD, Tekari A, Frauchiger DA, Krismer A, Benneker LM, Gantenbein B (2017) Efficient non-viral transfection of primary intervertebral disc cells by electroporation for tissue engineering application. Tissue Eng Part C Methods 23(1):30-37 doi: 10.1089/ten.TEC.2016.0355

Selected Conference Contributions

1. Frauchiger DA, Heeb S, Wöltie M, Benneker LM, Gantenbein B. Proliferation and differentiation on engineered silk scaffolds: From MSC towards NP-like cells. AOSpine Masters Symposium-Novel and Emerging Medicine. Bern. 2017.
2. May RD, Frauchiger DA, Benneker LM, Gantenbein B. Comparison of gene expression of discs from Diffuse Idiopathic Skeletal Hyperostosis (DISH) and healthy (trauma) patient. AOSpine Masters Symposium-Novel and Emerging technologies in Translational Medicine. Bern. 2017.
3. Bakirci E, Guenat O, Hugi A, Ahmad SS, Kohl S, Gantenbein B. Optimization of 3D printed hydrogels with primary cells for tissue engineering. AOSpine Masters Symposium-Novel and Emerging Technologies in Translational Medicine. Bern. 2017.
4. Frauchiger DA, May RD, Koch AK, Benneker LM, Gantenbein B. SPECIAL EMPHASIS POSTER: Real-time monitoring of glucose consumption of intervertebral disc cells in 3D culture. Proceedings of ISSLS Meeting. Athens, Greece, 28 May - 2 June. 2017.
5. Krismer A, Geissberger C, Bakirci E, Cabra R, Kohl S, Gantenbein B. (2017) Strain-controlled organ culture of bone-ligament-bone human-derived anterior cruciate ligaments - an ex-vivo model to investigate degenerative and regenerative therapy. Proceedings of TERMIS-EU Chapter Meeting. Davos. 2017.
6. Frauchiger DA, Tekari A, Benneker LM, Sakai D, Gantenbein B. The Fate of Allogeneic Injected Angiopoietin-1 Receptor Tie2+ Cells in Intervertebral Disc Organ Culture. Proceedings of the ORS. San Diego, USA. 2017.
7. May RD, Tekari A, Chan SCW, Frauchiger DA, Benneker LM, Gantenbein B. The Natural Expression of BMP Antagonists in Intervertebral Disc Cells. Proceedings of the ORS. San Diego, USA. 2017.