Tissue and Organ Mechanobiology

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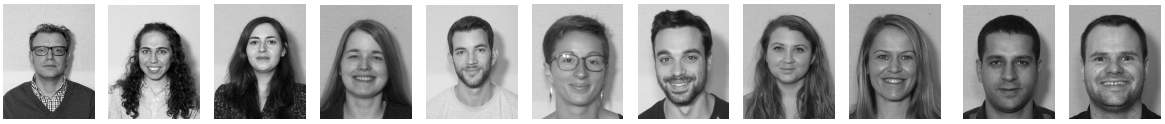

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Research Profile

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 is currently being investigated for IVD repair. Here, the TOM group investigated into new growth-factor-enriched silk, which is produced from genetically transduced silk worms (*Bombyx mori*), which embed the growth factor of interest directly into the silk. The new 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 fibrin hydrogel. Therefore, 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 (Figure 1). These results were recently reported in the November issue of the "Orthopädische Nachrichten" in a special issue on low back pain. Daniela Frauchiger presented her data at the Annual Meeting of the German Spine Society in Hannover.

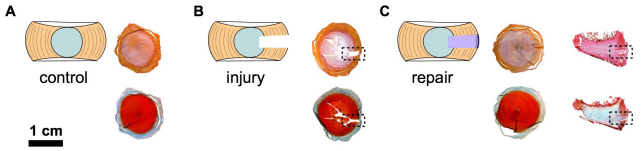

Figure 1. Histology of bovine IVDs after 14 days of *ex vivo* culture; top: Hematoxylin/Eosin, bottom: Safranin-O/Fast Green. **(A)** healthy control disc **(B)** injured IVD using a 2mm biopsy punch **(C)** IVD repaired with genipin-enhanced fibrin hydrogel and silk fleece-membrane composite. (Scale bar 100µm)

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. In organ culture experiments, we labelled isolated NPPC and injected them back into an artificially degenerated bovine IVD to study their behaviour in the native IVD environment (Figure 2). Future research is to understand how these cells can be isolated best and whether these cells can be maintained *in vitro* to regenerate the IVD.

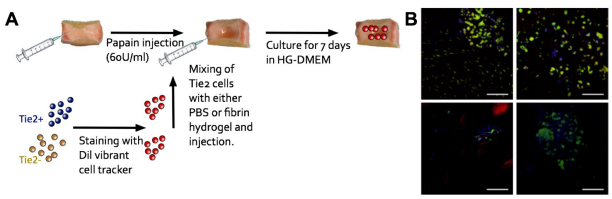

Figure 2. **(A)** nucleus pulposus progenitor cells (NPPC = Tie2+ cells) and Tie2- cells were isolated from bovine NP tissue and labelled with Vybrant™ DIL dye and seeded in fibrin hydrogel or phosphate buffered saline (PBS. Cells were then injected into a previously degenerated IVD cavity using papain. IVDs were then cultured for 7 days and stained with calcein AM and DAPI (live/dead assay). **(B)** Faith of injected NPPC and Tie2- cells was assessed using 3D stacks of confocal microscopy. NPPC injected with PBS (top row) and with fibrin hydrogel (bottom row). Live injected: yellow; dead injected: red ; live native: green; dead native: blue, (scale bar 100 µm).

Here, a selection of biomaterials and 3D cell culture systems might help to find suitable culture conditions to expand these cells.
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 (Figure 3).

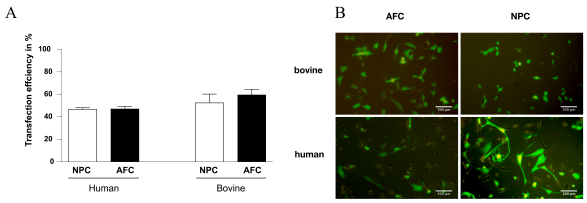

Figure 3. **(A)** Percentage of transfection efficiency of human (N = 4) and bovine (N =5) NPC and AFC as quantified by flow cytometry. The percentages of transfection efficiencies are (mean ± SEM): hNPC 46.7 ± 1.4 %, hAFC 47.1 ± 2.4 %, bNPC 52.44 ± 7.9 %, bAFC 59.6 ± 5.0 %. **(B)** Green fluorescent protein (GFP)-positive human and bovine annulus fibrosus (h- and bAFC) and nucleus pulposus cells (h- and bNPC) after 48 hours of transfection with pCMV6-AC-GFP were detected under a light microscope.

Biological Repair of the ruptured 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 (Inselspital) and the Sonnenhof clinic called Dynamic Intraligamentary Stabilization (DIS) which keeps ACL remnants 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-derived plasma (PRP) are of interest. The aim of our research was to investigate the use of collagen patches, the application of platelet rich plasma (PRP) and platelet rich fibrin (PRF) in combination with DIS to support regeneration of the ACL and to quantify the biological response. Furthermore, a novel bioreactor has been designed and realized to culture full human ACL (Figure 4). Here, first results were reported by mechanical stimulation of live ACLs for seven days.

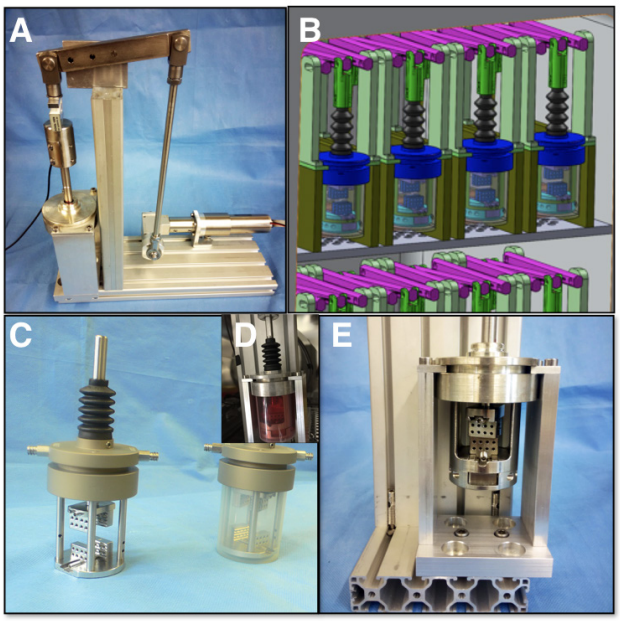

Figure 4. Strain-controlled bioreactor to culture human full ACL. **(A)** Side-view of ACL Bioreactor **(B)** CAD view of planned 4-stations bioreactor inside CO2-controlled incubator **(C)** Side-view of new culture chamber design **(D)** ACL in culture with culture medium **(E)** Close-up view of bioreactor set-up.

Original Peer-reviewed Journal Articles

1. Chooi WH, Chan SC, Gantenbein B, Chan BP (2016) Loading-Induced Heat-Shock Response in Bovine Intervertebral Disc Organ Culture. PLoS ONE 11(8):e0161615. doi: 10.1371/journal.pone.0161615.
2. Hoppe S, Wangler S, Aghayev E, Gantenbein B, Boger A, Benneker LM (2016) Reduction of cement leakage by sequential PMMA application in a vertebroplasty model. Eur Spine J 25(11):3450-3455 doi: 10.1007/s00586-015-3920-3
3. Schmocker AM, Khoushabi A, Frauchiger DA, Gantenbein B, Schizas C, Moser C, Bourban P-E, Pioletti D (2016) A photopolymerized poly-ethlyene-glycol composite hydrogel and surgical implanting tool for a nucleus pulposus replacement. Biomaterials 88: 110-119 doi: 10.1016/j.biomaterials.2016.02.015.
4. Tekari A, Chan SC, Sakai D, Grad S, Gantenbein B (2016) Angiopoietin-1 receptor Tie2 distinguishes multipotent differentiation capability in bovine coccygeal nucleus pulposus cells. Stem Cell Res Ther 7(1):75. doi: 10.1186/s13287-016-0337-9.

Selected Conference Contributions

1. Chooi WH, Chan SCW, Gantenbein B, Chan BP (2016) Compression Loading Induced Cellular Stress Response of Intervertebral Disc Cells in Organ Culture. Global Spine J 06(S 01):WST009. doi: 10.1055/s-0036-1582604
2. Frauchiger DA, Benneker LM, Roth E, Gantenbein B (2016) Annulus Fibrosus Repair using Genetically Engineered Silk and Genipin-Enhanced Fibringel. Global Spine J 06(S 01):WO001. doi: 10.1055/s-0036-1582588
3. Frauchiger DA, Tekari T, Benneker LM, Sakai D, Gantenbein B. (2016) The fate of Tie2+ nucleus pulposus progenitor cells injected into a papain degenerated organ culture model with and without hydrogel. Proceedings of ISSLS Meeting. Singapore, 15-20 May.
4. Krismer A, Cabra R, Kohl S, Ahmad SS, Gantenbein B. (2016) The Relative Gene Expression Profile of human Anterior versus Posterior Cruciate Ligament. Proceedings of the ORS Annual Meeting, Spineweek. Orlando, FL, 5-8 March.
5. Krismer A, Geissberger C, Thomi G, Cabra R, Kohl S, Ahmad SS, Gantenbein B. (2016) Strain-Controlled Organ Culture of Intact Human Anterior Cruciate Ligaments – An ***Ex-vivo*** Model to Investigate Degenerative and Regenerative Approaches. Proceedings of the ORS Annual Meeting. Orlando, FL, 5-8 March.
6. Tekari A, Chan SCW, Frauchiger DA, Benneker LM, Heini PF, Gantenbein B. (2016) The BMP2-variant L51P enhances the osteogenic differentiation of human mesenchymal stromal cells in the presence of intervertebral disc cells. Global Spine Congress/World Forum for the Disc. Dubai, 13-16 April.
7. Tekari A, Marazza A, Benneker LM, Gantenbein B. (2016) Inhibition of ERK pathway restores the discogenic phenotype of inflamed intervertebral disc cells. Proceedings of ISSLS Meeting, Spineweek. Singapore, 15-20 May.
8. Tekari A, May RD, Frauchiger DA, Sebald HJ, Benneker LM, Gantenbein B. (2016) The osteogenic differentiation of mesenchymal stromal cells is enhanced by the BMP2 variant L51P in the presence of intervertebral disc-derived cells. eCM XVII: Stem cells, Bone Fixation, Repair & Regeneration. Davos, Switzerland, 20th – 23rd June 2016.