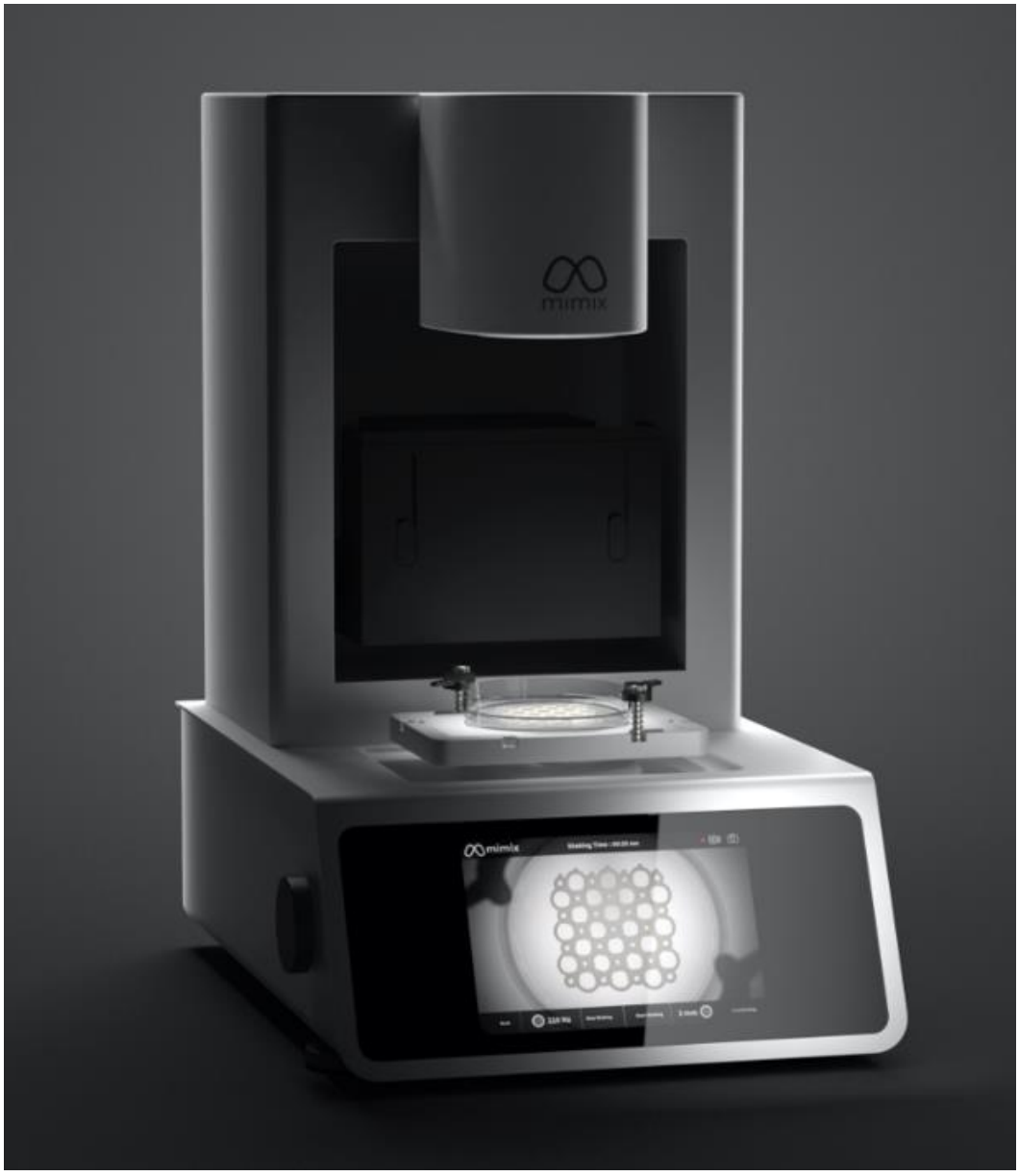
Research Institute Davos

ARI Activity Report 2020



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1 Introduction

Dear friends of AO Research Institute Davos (ARI),

For several reasons 2020 will be remembered as a very special year in the AO Research Institute Davos's history. This last year was unprecedented within my life and most of our lives with the COVID 19 pandemic. It has massively changed our routine of the last years. There were some major changes in virtual networking and meetings, yet unfortunately also some detriment to how we interact, as people need face to face physical meetings for our well being and the virtual meetings are not able to replace this most basic human nature. As this continues I hope this will not be detrimental to our strong team spirit. Many of us have lost friends and even family in this time and many of us who have families abroad have not been able to visit them and this has been very stressful.

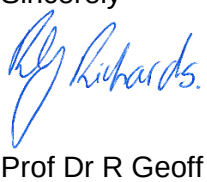
Due to the first lock down almost all practical work on projects had to be stopped and no new projects were started in the labs for about 3 months. The opportunity to focus on writing scientific publications was taken and resulted in a record number of papers with a new record in impact factor for 2020. After the restart of the practical work in the labs in June with limited capacity the time was very busy until the end of the year, also due to the fact that we still follow strict rules on maximum number of people present in one room. Nevertheless, most projects were back on track by the end of the year. Our SQS routine audit was online for the first time ever, but we passed for ARI (ISO 9001, EN ISO 13485) with no major deviations. The planned Swissmedic GLP certification routine inspection (November 2020) was postponed due to the pandemic.

On the Dec 15th, 2020 Mimix (ARI's partner, licencing ARI Intellectual property) launched the first acoustic bioprinter in the world, named CymatiX on the market (front cover ARI Activity Report). Another highlight was that the Tech-file for the ARI biphasic plate was submitted to notified body for CE certification in October. Development of the ARI Fracture Monitor showed significant progress in various aspects of the design process. All components relevant for the CE registrational clinical trial (implantable sensor with embedded software and smartphone application) are approaching design freeze to complete Phase II of the development process and obtain design output. A first version of the cloud environment for data collection and data access with minimal functionality went live.

It has been an extremely strange year where it has been hard to direct from an attic at home many days and nights of the week. I have missed meeting new fellows and learning their ambitions in life, missed time with the long-term team and missed our conferences where we can shine with our presentations of our work. Despite this I wish to congratulate the whole team on acquiring 3.16 million francs extramural funding, publishing 116 papers while even increasing in impact factor (4.4, well above the average in our fields).

Thank you all in ARI and our partners in the AO Foundation for your contributions to these great achievements. I also thank the volunteer AO network for their clinical advice to our projects, enthusiasm, motivation and time. Finally thanks to the ARI AC, despite not meeting in 2020 keeping close tabs and connecting with us.

Sincerely



Prof Dr R Geoff Richards FLSW, FBSE, FIOR
Director AO Research Institute Davos (ARI)

2 ARI Purpose / Goals / Outlook

Purpose

In its work to further the AO Foundation's mission (promoting excellence in patient care and outcomes in trauma and musculoskeletal disorders), ARI has the purpose to advance patient care through innovative orthopedic R&D.

Orthopedics concerns musculoskeletal, spine and cranio-maxillo-facial trauma, degenerative musculoskeletal diseases, infections, and congenital disorders.

Goals

- Contribute to high quality applied preclinical research and development (exploratory and translational) focused towards clinical applications/solutions.
- Investigate and improve the performance of surgical procedures, devices and substances.
- Foster a close relationship with the AO medical community, academic societies, and universities.
- Provide research environment- / research mentorship / research support for AO clinicians.

Goal Achievements

- Development & translation of our unique smart surgery concepts: AO Fracture Monitor **In Progress** - in 2020 this continued in the preparatory work for regulatory approval)
- Establishment of the first European SPF sheep facility (AO Specific Pathogen Free Sheep) **In Progress** - in 2020 the building of this and a Biogas facility to produce green energy was initiated, despite many setbacks due to being built upon one of Davos's old rubbish landfill sites, which added substantial costs to the building's foundation costs)
- Strengthen and advance our research activities in patient diagnostics and stratification **In Progress**
- Support Mimix Biotherapeutics in valorization of ARI-based biofabrication technology **Achieved** – With the strong support of ARI (and the AO Technology Transfer Office), Mimix launched the first acoustic bioprinter, named CymatiX, on the market on Dec 15th, 2020.

Rolling Outlook ARI goals (3 years 2021-2023):

- Development & translation of our unique smart surgery concepts: AO Fracture Monitor
- Establishment of the first European SPF sheep facility (AO Specific Pathogen Free Sheep)
- Development and translation of the ARI biphasic plate
- Strengthen and advance our research activities in patient diagnostics and stratification
- Support Mimix Biotherapeutics in R&D for their valorization of ARI-based biofabrication technology

Rolling Outlook ARI (next 5 years >2026)

- Maintain world-class research level and nurture in-house talents for long-term innovation
- Support AO with cutting edge R&D for clinical problems
- Continue developing ARI technology portfolio. Translate / valorize ARI innovations together with AO technology Transfer office of the AO Innovation Translation Centre (AO ITC)
- Maintain our world-class certifications (ISO, AAALAC, GLP).
- Continue to develop our 3D polymer printing & bioprinting technologies.
- Strengthen project based research within the ARI
- Strengthen our ARI Focus Areas
- Nurture our scientific networks (e.g. ARI collaborative research consortium), global societies (e.g. ORS, TERMIS, ICORS) and European societies we work with (ESB-Biomaterials, ESB-Biomechanics, EORS, TERMIS-EU).

3 Funding Summary

Income Statement	2019 Actual		2020 Actual	
in CHF '000	abs	%	abs	%
AO Foundation Contribution	10'519	74%	9'318	73%
3rd party Income	2'295	16%	2'540	20%
AO Intercompany	1'387	10%	944	7%
Total Income	**14'201**	**100%**	**12'803**	**100%**
AOTrauma *	3'846	28%	3'528	28%
AOSpine*	752	6%	456	4%
AOCMF *	746	5%	648	5%
AOVET *	75	1%	20	0%
AOTC *	596	4%	306	2%
AO Fundamental Research	2'166	16%	1'960	16%
AO Foundation *	3'112	23%	3'125	25%
3rd party projects	2'295	17%	2'540	20%
Total Expenses	**13'590**	**100%**	**12'583**	**100%**
Net Result	**612**		**219**	

* incl. AO Intercompany

The decline of the AO Foundation contribution by CHF 46 K compared to budget is caused by a decrease of operational activities due to the Covid-19 pandemic.

'3rd Party Income' amounted to CHF 2,540 K and was 14% (CHF 427 K) below budget and 11% (CHF 245 K) above previous year. The reason for the variance was a shift of income generated from the Development Incubator projects, originally budgeted under "3rd party income", to "Intercompany income" in the current year. As a total income remained on a par with budget.

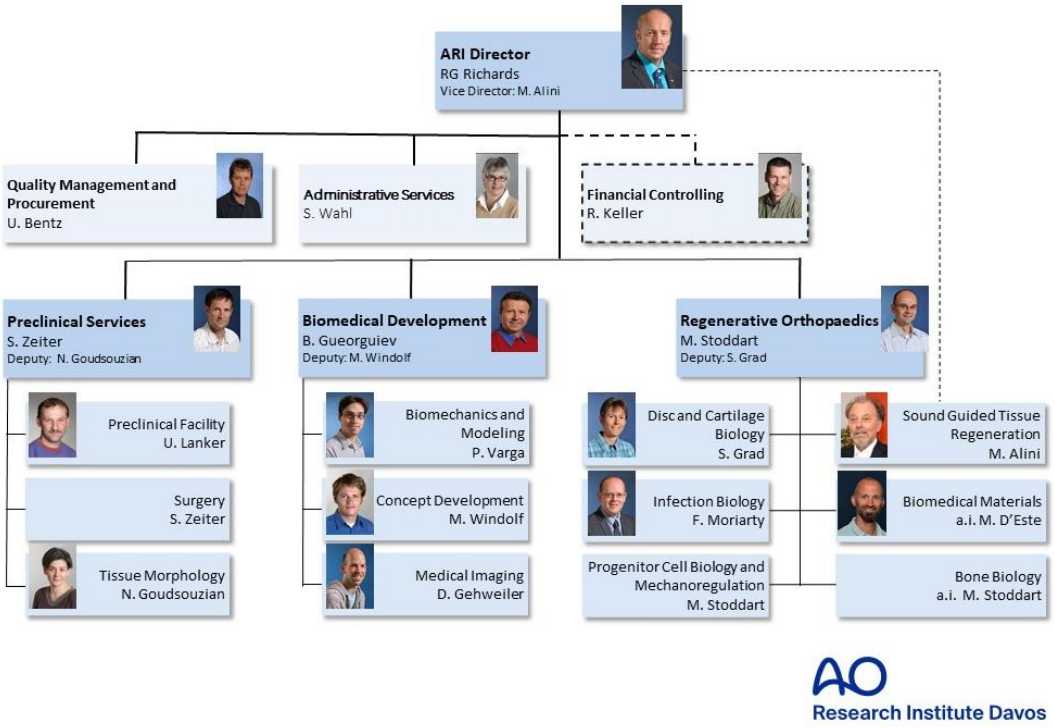
With regards to the split of the 'Total Expenses' by organizational unit, 'Regenerative Orthopaedics' had the highest share with 44% followed by 'Biomedical Development' and 'Preclinical Services' with 22% and 20% respectively. The underspend versus budget of "Biomedical Development' amounted to CHF 173 K (6%) and was mainly caused by leaving staff not fully replaced and lower travel expenses due to Covid-19 restrictions. The underspend in 'Management' of CHF 52 K (4%) versus budget resulted mainly from lower travel expenses for non-employees also due to given Covid-19 regulations. The variance versus budget in 'Fellowships' (CHF 105 K / 20%) is driven by a higher number of Internships and Fellows.

From a cost type point of view, the main categories were 'Personnel Expenses' with 71%, followed by 'Material Expenses' with 11% and 'Scientific & Regional Expenses' with 5% of total costs.

Overall, a positive 'Net Result' of CHF 219 K was achieved compared to a balanced budget.

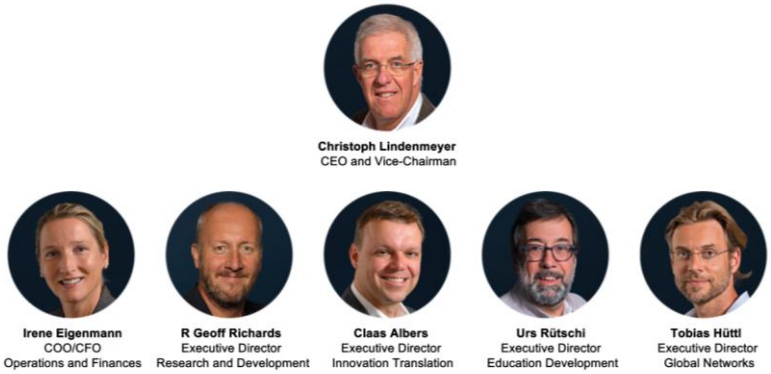
4 Research Structure & Advisory Committees

4.1 AO Research Institute Davos (ARI) Organigram



In 2020, Regenerative Orthopaedics program leader deputy Prof David Eglin was appointed as full-time professor at Ecoles des Mines Saint Etienne, IMT, France. Therefore, Sibylle Grad agreed to take over again the deputy function. Matteo D'Este took over *ad interim* leadership of Biomedical Materials. Prof. Mauro Alini's Focus Area was renamed Sound Guided Tissue Regeneration (former Organ Models).

4.2 AO Foundation Executive Committee (AOEC)



After thorough analysis of the current AO Foundation leadership structure supported by an external coach, Geoff Richards was appointed Executive Director Research and Development globally, which now comprises not only ARI, but shortly R&D undertaken in the Global Networks of the AO Foundation (not including AO North America or small regional start up grants). The Executive Committee is a much smaller and focused group compared to the former AO Executive Management team (AOEM), which had nine executive members and four permanent guests. It focuses much more on strategy than on daily business compared to the former AOEM.

4.3 AO Foundation R&D Platform

The AO R&D Platform supports the active exchange and mutual discussion about strategies of the AO units with respect to their related goals in R&D. It supports the AO Foundation Board (AOFB) in defining general strategic areas and their implementation in an advisory function. It ensures that relevant activities are in line with the AO Mission and strategies as defined by the AOFB. All research stakeholders are finally accountable to the AOFB. The AO R&D Platform will monitor, review and further develop the strategies defining clinical needs (in general) and their implementation on behalf of the AOFB in an advisory capacity. It has no funding and decision authority. Educational aspects will be treated through the existing Education Platform and innovation and valorization aspects through the existing Innovation Platform. The R&D Platform is represented on the AOEC by the Director of the ARI. The R&D expert of the AOFB is the Chair of the R&D Platform, currently Prof Anita Ignatius, Director and Chair of the Trauma Research Center Ulm (ZTF), University Hospital Ulm, University of Ulm, Germany. Due to the COVID 19 Pandemic, no R&D Platform meetings were held in 2020. It was decided not to hold virtual meetings for these meetings.

4.4 AO Research Institute Davos Advisory Committee

The AO Research Institute Davos (ARI) Advisory Committee (ARI AC) provides operational and strategic scientific advice to the ARI on behalf of the AO Foundation Board. ARI AC acts as both a sounding board and sparring partner for the Director and scientists of the ARI.

The ARI AC's tasks and responsibilities include advising ARI on:

- Portfolio of competences (skills of personnel and type of equipment)
- Strategy and priority setting for direct funds of ARI
- Exploratory collaborative research program(s)
- Business development and initial advice on technology transfer
- Regulatory issues
- Use of ARI funds
- Advancement of the ARI capabilities, to assure the efficient use of the infrastructure

The ARI AC comprises the following members:

- Prof Theodore Miclau (Chair, represents the ARI AC and member of the AO R&D Platform), Orthopedic Trauma Institute, USA
- Prof Chris Evans, Mayo Clinic, USA
- Prof Brian Johnstone, Oregon Health and Science University, USA
- Prof Joost de Bruijn, University of Twente, the Netherlands

Due to the COVID 19 Pandemic, no ARI AC meetings were held in 2020. It was decided not to hold virtual meetings for these meetings.

5 ARI Teams / Personnel

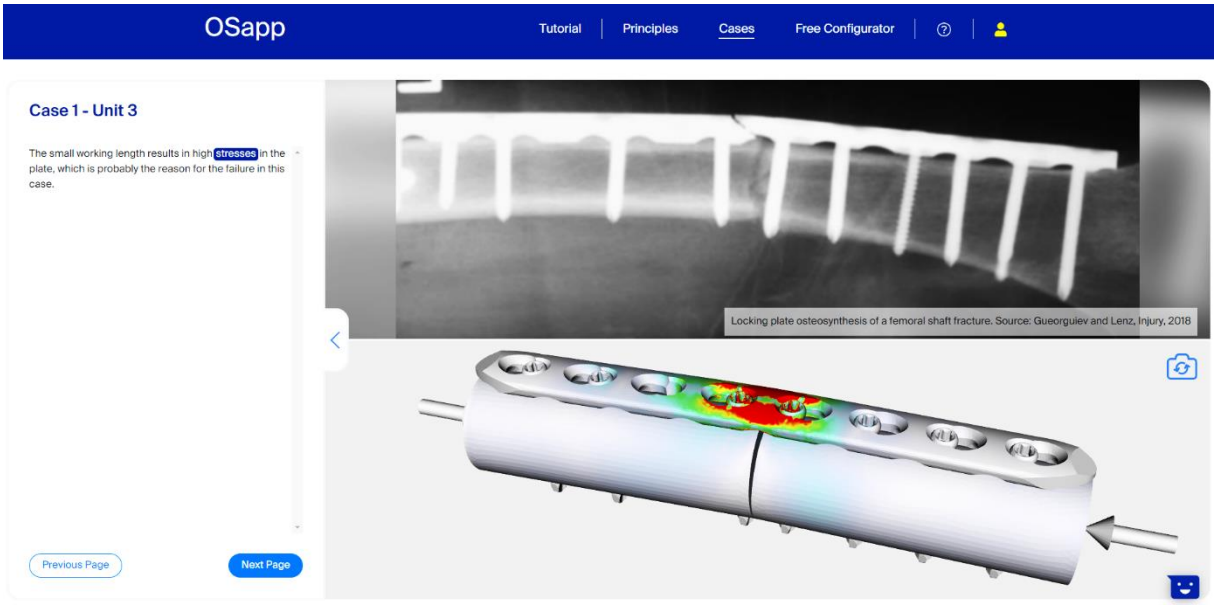
5.1 Biomedical Development Program

Program Leader: Boyko Gueorguiev-Rüegg, Deputy: Markus Windolf

Team Members: David Ambühl, Paolo Antonacci, Jan Barcik, Amirsiavosh Bashardoust, Jan Buschbaum, Jan Caspar, Daniel Ciric, Carolin Danker, Manuela Ernst, Dominic Gehweiler, Ladina Hofmann-Fliri, Lukas Kamer, Manuel Knecht, Dominic Mischler, Karen Mys, Hansrudi Noser, Magdalena Remppis, Ronald Schwyn, Flurin Spiller, Peter Varga, Viktor Varjas, Dieter Wahl, Ivan Zderic, Erich Zweifel

Fellows: Jan Dauwe, Torsten Pastor, Guillermo Sanchez, Jana Schader, Aleksandar Stefanov

Supporting the in-house processes for development and design of medical devices according to EN ISO 13485 and running advanced projects in close collaboration with clinical, scientific and industrial partners, as well as with the AO clinical divisions and the AO Innovation Translation Center, the Biomedical Development Program offers extensive know-how, expertise and experience in the fields of biomechanical testing and computational analyses to improve patient care.
A variety of clinical problems are addressed by development of new concepts, approaches, tools and novel implant systems for surgical applications and research in traumatology and orthopedics. The process of finding optimal solutions to clinical questions is enhanced by capabilities ranging from *in silico* methods to very well-equipped anatomical labs for quick and effective hands-on work when an anatomical environment is required. Specifically, tailored test procedures with implementation of supplemental X-rays, video and motion tracking systems are applied in diverse experiments on fracture fixation and joint reconstruction. Advancing with state-of-the-art technologies, powerful numerical methods and comprehensive tools for virtual simulations are integrated to answer various questions with special reference to biomechanical performance of bone-implant constructs. Modalities for medical imaging, processing, and analysis, including CT scanners with a wide range of resolutions and scanned volumes, are interlinked to account for increasingly sophisticated demands for morphological investigations, extract statistical and individual information from medical image data and extend the knowledge on variations of biomechanical bone characteristics and their role in persisting clinical problems. The capabilities of the Program are completed by the Prototype Workshop offering rapid and high-quality manufacturing of devices, tools and implants.



OSapp – virtual osteosynthesis tool for surgical education by demonstrating biomechanical principles of implant fixations.

5.2 Preclinical Services

Program Leader: Stephan Zeiter, Deputy: Urban Lanker

Team Members: Daniel Arens, Mauro Bluvol, Carmen Brazerol, Tim Buchholz, Caroline Constant, Peter Erb, Loris Faoro, Pierina Faoro, Andrea Furter, Nora Goudsouzian, Nilo Hämmerl, Maria Hildebrand, Reto Müller, Dirk Nehrbass, Dominic Perren, Monika Schneider

Fellows: Lena Gens, Brenna Pugliese, Hella Schwegler, Mai Thanh Vo, Charlotte Wittmann

Student Externs: He Chang, Lotta Reimann, Maja Strunk

Preclinical Services succeeded in supporting the ARI's mission by performing a multitude of *in vivo* studies successfully. We were able to continue working on over 20 studies in the field of regenerative orthopedics, implant associated infections and implant development involving different models in mice, rats, rabbits, and sheep. Due to consistent implementation of the AO's protection concept, no study had to be interrupted prematurely and therefore no animals had to be euthanized due to the pandemic measures. During 2020, three studies were successfully conducted under GLP regulations as a part of the legal approval process - two with external partners and one testing a device developed within the ARI.
Training of students, fellows, and scientists in histological techniques necessary for ongoing projects continued, but needed to be adapted to the pandemic situation e.g. teaching of histopathological evaluation took place remotely by streaming.
As part of a constant effort to improve, the entrance to the Biosafety Level 2 area used for housing animals enrolled in infection-related studies was rebuilt ensuring a more efficient workflow. The construction of the SPF (specific pathogen free) sheep facility was initiated, which will house the first European SPF sheep flock in a few years.
Two members of the team completed the next steps in their respective careers with Dr Caroline Constant becoming a Diplomate of the American College of Veterinary Surgeons - Large Animal (DACVS-LA) as well as finishing her Master's degree in Engineering and Clinical Science and Tim Buchholz received his doctoral degree in veterinary medicine from the university of Bern. Enabling and supporting such personal advancements fosters expert knowledge within the team and guarantees the high quality of our work and teaching. In line with this, we continued our efforts to educate the next generation of veterinarians by enabling 3 fellows and 3 students to gain an insight and on-hands experience with preclinical research.
For all *in vivo* studies performed at Preclinical Services, we aim for the highest standard of animal welfare, quality of generated data and occupational health and safety through consequent implementation of the 3R principles and adherence to the regulations of our GLP, AAALAC and ISO 9001:2015 accreditations. In this regard, in order to constantly challenging ourselves to improve, two studies were conducted looking at the refinement of analgesic protocols for different surgeries.
Being active in different societies i.e. the Preclinical Model Section at the Orthopaedic Research Society (ORS), the European College of Laboratory Animal Medicine (ECLAM), the Federation for Laboratory Animal Science Associations (FELASA) and the Swiss Laboratory Animal Science Association (SGV), ensures that we pursue best in class policies in the sensitive area of animal models. Further, continued membership to pathology societies such as the European Society of Toxicologic Pathology (ESTP) and the Society of Toxicology Pathology (STP) keeps knowledge in the field of analysis up-to-date.

5.3 Regenerative Orthopaedics

Program Leader: Martin Stoddart, Deputy: Sibylle Grad

Team Members: Gion Alig, Mauro Alini (Vice Director), Angela Armiento, Cecilia Bärtschi, Romain Bagnol, Valentina Basoli, Yamina Baumgartner, Elena Della Bella, Matteo D'Este, Nicolas Devantay, Nunzia Di Luise, Nicola Di Marzio, Janick Eglauf, David Eglin, Priscilla Füllemann, Pamela Furlong, Géraldine Guex, Surya Häne, Johannes Hasler, Phelipe Hatt, Joseph Hintermann, Marloes Hofstee, Hermann Kasper, Iris Keller-Stoddart, Nadine Kluser, William Lackington, Yann Ladner, Wenyue Li, Zhen Li, Junxuan Ma, Ursula Menzel, Gregor Miklosic, Graziana Monaco, Fintan Moriarty, Andrea Nüesch, Marianna Peroglio, Robert Peter, Virginia Post, Aapo Ristaniemi, Stijn Rotman, Andrea Schwab, Amra Sercovic, Tiziano Serra, Claudia Siverino, Astrid Soubrier, Christoph Sprecher, Flurina Staubli, Eric Sumrall, Keith Thompson, Win-Hon Trinh, Letizia Vainieri, Daphne van der Heide, Andrea Vernengo, Sophie Verrier, Alexandra Wallimann, Sylvie Wirth, Taiyo Yamamoto, Reihane Ziadlou, Daniele Zuncheddu

Fellows: Paras Ahmad, Susanne Bärtl, Shangbin Cui, Jan Gewiess, Maria Antonia Gomez Sierra, Wei Hao, Walker Magrath, Céline Tourbier, Katie Young, Phenghui Zhang

Guests: Melanie Acosta, Preeta Ananthanarayanan, Talita Aygün, Teresa Brose, Yanan Fu, Peng Guo, Chen Guoliang, Edera Marcello, Jiang Nan, Babak Saravi, Lisa Sturm, Hidaka Takuya, Tim Wesdorp

2020 was a year of big changes in the Regenerative Orthopaedics Program. After 20 hugely successful years of leadership under Prof Mauro Alini, Prof Martin Stoddart became the head of the Musculoskeletal Regeneration program. To reflect this change, and to reflect changes in research direction that were started many years ago, the program name was changed to Regenerative Orthopaedics and the program was restructured. Focus Area names were changed to better emphasize the research carried out by the teams and we welcomed the Infection Biology team to the Program. Furthermore, a new Focus Area based on Sound Induced Morphogenesis was established and provided on opportunity to retain the vast experience of Prof Alini as its leader. The Regenerative Orthopaedics Program develops biological approaches addressing pathologies of the musculoskeletal system, with a focus on bone, cartilage, and intervertebral disc. The ultimate goals are to identify strategies for prevention or attenuation of degenerative processes and to re-establish tissue functionality.

Bone Biology Focus Area

Bone healing in response to fracture involves a complex sequence of dynamic events, directed by numerous different cell types and growth factors. A critical factor for bone repair is the maintenance, or effective restoration, of an adequate blood supply, which is necessary to provide the damaged tissue with oxygen, nutrients, and growth factors, as well as immune cells and mesenchymal stem cells required to repair the damage and induce new bone formation. Although bone generally has a high regenerative capacity, in some cases this inherent bone healing is compromised, which results in delaying healing or non-union of the bone fracture with increased health care costs and reduced quality of life issues for affected patients. While a variety of risk factors have been identified that predispose to an increased risk of developing delayed bone healing or non-union, it is currently not possible to identify specific at-risk patients at an early stage. Using *in vitro* and *in vivo* techniques, the aim of the Bone Regeneration Focus Area is to gain a greater understanding of the immunoregulation, cellular interactions and mediators and underlying mechanoregulation. By determining how cells such as immune cells, mesenchymal stem cells and endothelial cells normally interact during the repair process, and how this process is altered during impaired healing, we can then identify key events in the healing process. Our goal is to use tissue engineering and regenerative medicine approaches to promote bone healing, aimed at restoring bone integrity and its effective biomechanical properties.

Disc and Cartilage Biology Focus Area

We aim at investigating mechanisms that lead to intervertebral disc (IVD) damage and evaluating novel biological treatment methods for IVD repair and regeneration. Acute and chronic damage to the IVD are major causes of low back pain. However, factors that contribute to loss of IVD function and the underlying pathophysiology are still poorly understood. We have established a whole IVD organ culture system with the ability to maintain entire discs with the endplates for several weeks under controlled nutrient and mechanical loading conditions. Within this bioreactor, the beneficial or detrimental effects of nutrition, mechanical forces, and/or biochemical factors on disc cell viability and metabolic activity are investigated. We have developed various defect and degeneration models, allowing us to design and evaluate appropriate biological treatment strategies. These include implantation of cells, delivery of anabolic, anti-catabolic or anti-inflammatory molecules, biomaterials, or a combination thereof. Data from *ex vivo* models are also correlated to *in vivo* observations to identify molecular markers of IVD damage or degeneration.

To study the potential of new therapies for articular cartilage repair and regeneration, a bioreactor system applying multiaxial load to tissue-engineered constructs or osteo-chondral explants has been established. The bioreactor mimics the load and motion characteristics of an articulating joint. Chondral and osteochondral defect and disease models enable us to test tailored treatments under physiologically relevant mechanically loaded ex-vivo conditions. Cell- and material-based therapies as well as chondrogenic or anti-inflammatory factors are under investigation for cartilage repair and regeneration.

Biomedical Materials Focus Area

The Biomedical Materials focus area is committed to the design of advanced biomaterials and the development of manufacturing technologies to achieve improved patient care and outcome in musculoskeletal disorders. Biomaterials for skeletal repair can provide structural and mechanical features for the filling of defects, but also be carrier for drugs, cells and biological factors. One of our goals is the development of 3D structures for bone and cartilage tissue engineering, using tailored polymers and composites manufactured with additive manufacturing processes. Our experience lies in the design of biocompatible, biodegradable macromolecular networks and their processing with controlled architecture and embedded biologics. A second field of research investigates the preparation of hyaluronan-based biomaterials which can be used to deliver drugs and cells. These injectable biodegradable materials have considerable potential in tissues repair and drug delivery. We are also developing innovative technologies for the structuration and assembly of tissue-like matrices aiming to mimic for example, biological matrix mechanical and structural anisotropy.

Progenitor Cell biology and Mechanoregulation Focus Area

The Progenitor Cell biology and Focus area is particularly interested in stem cell therapies for bone and cartilage that could be applied within a clinical setting. We have been identifying predictive markers of donor variation with the aim to predictively identify the potency of cells from individual donors. In the search for biomarkers to determine patient specific healing potential, extracellular vesicles and non-coding RNA sequences such as miRNA are increasingly being used as a diagnostic and therapeutic tool. The development of a serum-based biomarker approach would dramatically improve patient specific clinical decisions. We also aim to investigate the role of mechanical and soluble factors in the activation of mesenchymal stem cells, and the promotion of differentiation and tissue repair. Mechanical forces can be applied by way of rehabilitation protocols and are able to modify stem cell and macrophage function. Such studies are forming the basis of the emerging field of regenerative rehabilitation. In addition to the effect of load on direct differentiation, it is known that biomechanical stimulation can modulate the cell secretome. Investigating these changes could lead to the identification of new targets, that may be present during articulation. This offers new avenues for potential clinical therapies.

Infection Biology Focus Area

Fracture-related infection (FRI) remains one of the most challenging complications in orthopedic and musculoskeletal trauma surgery. FRI has been convincingly shown to delay healing, worsen functional outcome, and incur significant socio-economic costs. Antibiotic prophylaxis, wound debridement, and postsurgical care can reduce, but not prevent, the incidence of these infections and so novel interventional strategies are required. The musculoskeletal infection team work on *in vitro*, *in vivo* and *ex vivo* studies to better understand, diagnose, prevent, and treat FRI.

A significant portion of the work performed by the Infection Biology team involves collaboration with the preclinical services team in ARI to model FRI in a complex living system and provide robust evaluation of the new interventional technologies under development such as antibiotic loaded hydrogels. This expertise also extends to extramural studies performed with industrial partners to evaluate external innovations in the prevention and treatment of FRI prior to clinical implementation. In parallel to the preclinical *in vivo* evaluations, greater focus has been applied to the opportunities of working with human materials, either *in vitro* through basic cell culture studies and also in clinical studies with patients experiencing FRI. Through partnerships with clinician scientists in the AO network, we have gained access to biological materials from patients with FRI in an effort to more accurately study host pathogen interactions and microbiome studies, as two recent examples.

Sound Guided Tissue Regeneration Focus Area

Spatial patterns of cells, organoids, or inorganic particles can be forced on demand using acoustic surface standing waves, such as the Faraday waves. This technology allows tuning of parameters (sound frequency, amplitude, chamber shape) under contactless, fast and mild culture conditions, for morphologically relevant tissue generation. We call this method Sound Induced Morphogenesis (SIM). The Sound Guided Tissue Regeneration Focus Area uses SIM for morphogenesis induction and further explorations in the regenerative medicine and cell therapy fields. Main activities are articulated around the translation of innovative biofabrication technologies for the repair of musculoskeletal disorders and development of cutting-edge 3-D *in vitro* disease models for drug screening and personalized medicine. To enable that, we use our sound wave-based approach and other external fields (e.g. light, magnetic, electric) for contactless cell assembly and stimulation.

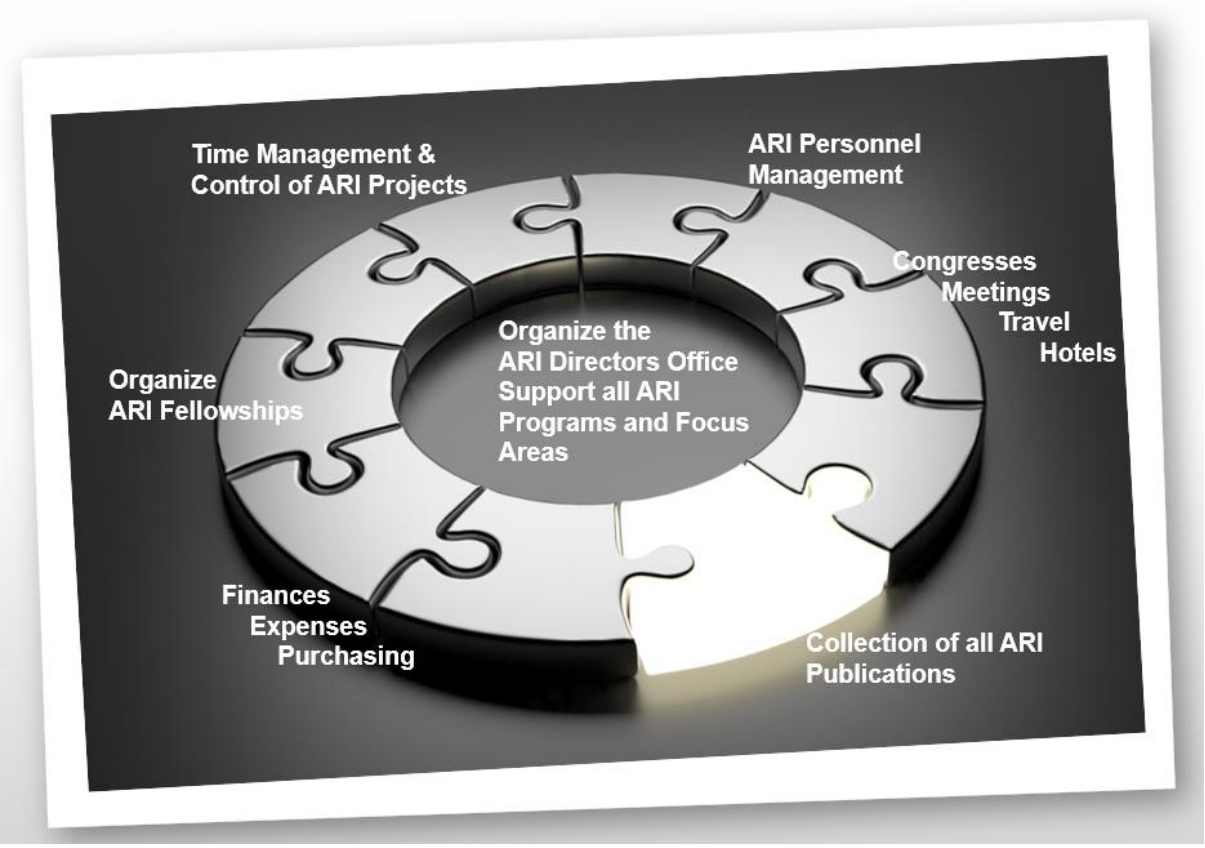
5.4 ARI Administrative Services

Manager: Sonia Wahl

Procurement: Ulrich Bentz

Team Members: Isabella Badrutt, Claudia Barblan, Simona Ciriello, Carla Escher, Gregor Müller, Sandra Steiner, Marisa Vivalda

The main goal of the ARI Administrative Services team is to provide an excellent service in all administration and organization fields of the ARI and to numerous AO Partners.



5.5 Operations standard and safety

Quality Manager: Ulrich Bentz

Successful 2020 routine audit of AO Research Institute Davos

From April 20 to 21, 2020, an external auditor from the SQS (Swiss Association for Quality and Management Systems; www.sqs.ch) audited ARI two days for the routine audit of the institute in an online audit. ARI has passed the routine audit 2020 without any non-conformities requiring immediate actions.

The entire AO Research Institute Davos is certified according to the international standard ISO 9001:2015.

The Focus Area Concept Development of the Biomedical Development Program are additionally certified to develop and test medical devices according to EN ISO 13485:2016 standard.

ARI is one of the very few academic research organizations to have achieved this certification.

AAALAC international accreditation of Preclinical facility

The Preclinical Facility was first accredited by AAALAC International in early 2013. The Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), is a private, no-profit organization that promotes the humane treatment of animals in science through voluntary accreditation and assessment programs. ARI is one of only 2 accredited institutions in Switzerland and the only accredited academic research institute in Switzerland. In November 2018, we received the third AAALAC international site visit, resulting in another 3-year accreditation.

GLP (Good Laboratory Practice)

ARI is listed as GLP compliant test facility since February 2016.
(https://www.anmeldestelle.admin.ch/chem/en/home/themen/gute-laborpraxis/pruefeinrichtungen.html).

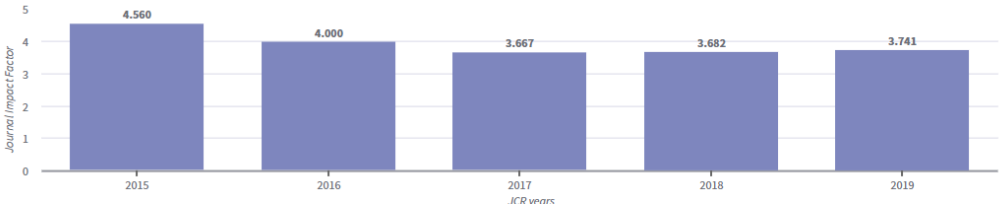
The second inspection took place in June 2018 and, on the 12 of October 2018, the Swiss Federal Office of Public Health renewed the statement of GLP compliance for the next 3 years. This is a major achievement for our institute after the AAALAC accreditation in 2013.
We are able to offer contract research services to all interested customers under GLP, especially if they want to get their medical devices approved by the FDA. Indeed, since the achievement of the GLP certification, all major commercial studies have been conducted under GLP (excluding pilot studies).
The planned Swissmedic inspection from November 2020 was postponed due to the Pandemic to May 2021.

6 eCM Journal & eCM periodical

Editor-in-Chief: R Geoff Richards
Production Editors: Simona Ciriello, Iolo ap Gwynn (external)
Webmaster, Web Editors: Simona Ciriello, R Geoff Richards, Martin Stoddart

eCM Journal (Eur Cell Mater) was the first Not-for-Profit, open access scientific peer-reviewed journal arguably in the world and certainly in the musculoskeletal field (initiated in 1999, implemented with the launch of the first volume in January 2001). It was created by scientists for scientists and is still run fully by scientists. eCM Journal is published by the AO Research Institute Davos (ARI), a Not-for-Profit foundation in Switzerland.
eCM is an Open Access journal: all publications have been immediately freely available upon publication since the journal start. Articles are freely accessible to the public without any embargo period, irrespective of who funded the research. This is equivalent to the new term "Gold Open Access" where articles are immediately available for others to read, download and share. In 2000, reviewing the first papers before launch of published papers in 2001, eCM initiated a transparent review process, naming reviewers within all published manuscripts. Reviewers also have a transparent route for becoming an official listed eCM reviewer (member of the eCM International Review Panel).

In June 2020, Journal Citation Reports (JCR) announced eCM's 2019 Impact factor (IF) to be 3.741. JCR 5-year Impact Factor: 4.31.



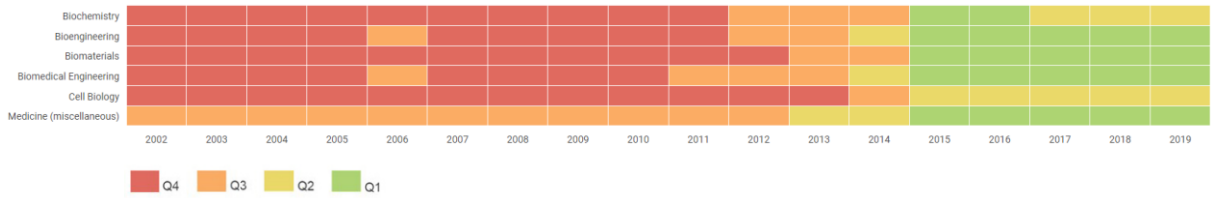
eCM is listed in the following JCR categories:

JCR ® Category	Rank in Category	Quartile in Category
CELL & TISSUE ENGINEERING	15 of 29	Q3
ENGINEERING, BIOMEDICAL	21 of 87	Q1
MATERIALS SCIENCE, BIOMATERIALS	16 of 38	Q2
ORTHOPEDICS	11 of 82	Q1

Scopus CiteScore 2019: 6.0. The Scopus CiteScore 2019 measures the average number of citations received in 2016-2019 to documents published in the same time frame.

Category	Rank	Percentile
Engineering └ Biomedical Engineering	#48/225	78th
Materials Science └ Biomaterials	#24/100	76th
Biochemistry, Genetics and Molecular Biology └ Biochemistry	#100/407	75th
Chemical Engineering └ Bioengineering	#41/148	72nd
Biochemistry, Genetics and Molecular Biology └ Cell Biology	#93/274	66th

SJR H index: 79.
The SCImago Journal Rank (SJR) indicator is a measure of the scientific influence of scholarly journals that accounts for both the number of citations received by a journal and the importance or prestige of the journals where the citations come from. A journal's SJR is a numeric value indicating the average number of weighted citations received during a selected year per document published in that journal during the previous three years. Higher SJR values are meant to indicate greater journal prestige.



eCM publishes preclinical research that has clinical relevance in the musculoskeletal field (Orthopaedics, Trauma, Maxillofacial (including dental) and Spine). eCM's definition of the musculoskeletal field includes bone, teeth, cartilage, intervertebral discs, skeletal muscle (not smooth or cardiac muscle), tendons and ligaments (it does not include the spinal cord or neural tissues).
Within the musculoskeletal field areas include:

- Assessment of materials for biomedical use
- Tissue Engineering and Regenerative Medicine (TERM)
- Structure, function, biology and biomechanics of connective and mineralized tissues
- Stem and Progenitor Cells
- Infection

From 2020, eCM reviewers can receive Publons credit for their review. They can track, verify and showcase their peer review and editorial contributions on Publons.

Addition to eCM team
Scientific Editors
Prof Denitsa Docheva, PhD
Experimentelle Unfallchirurgie, Klinik und Poliklinik für Unfallchirurgie, Regensburg, DE

PD Sibylle Grad, PhD
AO Research Institute Davos, Davos, CH

Ambassadors
eCM Ambassador to Eastern China 2019-2021
Prof Hong Wei Ouyang
Zhejiang University, Hangzhou, Zhejiang, China

eCM Ambassador to Southern China 2020-2022
Prof Yuxiao Lai
Director, Centre for Translational Medicine Research and Development, Shenzhen Institutes of Advanced Technology, Shenzhen, China

Special Issues launched in 2020
Tendons and Ligaments Special Issue: This Special Issue aims to deliver an up-to-date synopsis on tendon stem/progenitor cell biology under degenerative and regenerative conditions, cell-to-matrix/material interactions controlling cell behaviour, 3D niches/models, tendon engineering and biofabrication strategies as well as repair/regeneration models.

Dental Regenerative Biology Special Issue: This Special Issue intends to provide a comprehensive overview of how dental tissues respond to destructive insults, highlight the cues that are involved in their regeneration and report on the most recent developments in dental regeneration involving a combination of tissue engineering and stem-cell biology.

Bone Healing Special Issue: Although the regenerative capacity of bone is impressive, its failure to heal in certain circumstances continues to present clinical problems despite the high volume of multi-disciplinary research in this area. Approaches to improve bone formation in a clinically useful manner are drawn from many areas including biology, materials science, engineering, chemistry, physics and veterinary medicine.

Orthopaedic Infection Special Issue: Orthopaedic infections, including fracture-related infection (FRI), peri-prosthetic joint infection (PJI), septic arthritis and osteomyelitis, remain amongst the most challenging complications in orthopaedic and musculoskeletal surgery. These infections have been convincingly shown to delay healing, worsen functional outcome and incur significant socio-economic costs.

Ten good reasons for publishing a paper in eCM

World-wide Gold Open Access, authors retain copyright to their articles (CC-BY-SA).

1. eCM is a Not-for-Profit journal published by a Not-for-Profit foundation in Switzerland.
2. Rigorous open peer reviewing (reviewers have to request their name to be withheld). Reviewers can track, verify and showcase their peer review and editorial contributions on Publons.
3. Speed of publication.
4. Unique discussion with reviewers, as an integral section of the paper, allows sensible arguments to be included.
5. Scopus CiteScore **2019: 6.0** SJR **H index: 79. JCR Impact Factor 2019: 3.741**. **JCR 5-year Impact Factor: 4.31**.
6. Indexed in the Science Citation Index Expanded and Web of Science, DOAJ, ISSN, Scopus, SJR, Journal Citation Reports/Science Edition, Google Scholar, NCBI database, PubsHub and SHERPA/RoMEO databases. eCM articles can be searched directly from PubMed and China Knowledge Resource Integrated Database.
7. Digital archive of manuscripts through CLOCKSS and Europe PMC. eCM is a member of CROSSREF (Crossref Digital Object Identifiers (DOI:10.22203/eCM), tagged to article metadata).
8. Transparent route to becoming a member of the International Review Panel.
9. Created (and run) by scientists for the benefit of Science rather than profit.

eCM Open Access Not-for-Profit online periodical

eCM Periodical was initiated in 2017, previously run within eCM journal as eCM supplements. eCM Conference Online Periodical is not part of the eCM journal publication but is owned as a separate part of eCM. It hosts all eCM official society meeting abstracts along with other abstracts for various congresses as collections of combined individual meeting abstracts in PDF format. The individual abstracts within the abstract collections have been peer reviewed by the respective conference organizers. eCM Periodical has been recorded permanently in the ISSN Register, ISSN: 2522-235X from the ISSN International Centre. The abstract collections do not have a DOI, and the abstracts are not searchable on PubMed. eCM Conference Online Periodical was established to solve the long-standing problem of eCM supplements being used in the JCR/Clarivate Analytics calculation of eCM impact factor and, unfortunately, accounting for approximately 15% of eCM citable items.

6.1 eCM annual conference

Due to the Covid-19 pandemic, eCM XX: Biofabrication for Orthopaedics was postponed to December 8-11, 2021.

7 Institutional and Professional Relations

Geoff Richards is Director of the ARI since 2009 and has been at ARI since 1991. He has an appointment as full Professor at the Medical Faculty of Albert-Ludwigs University, Freiburg, Germany (since 2015). He has an honorary Professorship at Cardiff School of Biosciences, Cardiff University, Wales, GB (since 2007). He is a Distinguished Professor at The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China (since 2016). He is a Fellow of Biomaterials Science and Engineering (FBSE), Fellow of International Orthopaedic Research (FIOR). He was awarded honorary Fellow in 2019 of Aberystwyth University in Wales. In 2020 Geoff was elected Fellow of the Learned Society of Wales (FLSW). The Learned Society of Wales is the national academy for arts and sciences. He has Doctor Honoris Causa from the Technical University of Varna, Bulgaria. In 2017 Geoff co-founded of the International College of Fellows for Orthopaedic Research at the International Combined Orthopaedic Research Societies (ICORS), where he represents AO Foundation as a executive committee member. Geoff is cofounder and Editor-in-Chief of the Not-for-Profit open access eCM Journal and eCM periodical. He is an Associate Editor of the Journal of Orthopaedic Translation. He has Life Honorary Membership of the Swiss Society of Biomaterials. He is president (2019-2021) of TERMIS Global (Tissue Engineering & Regenerative Medicine International Society). He is a guest lecturer of the MSc Course Skeletal Repair at the Department Health Science and Technology (D-HEST) of the ETH Zurich. Geoff is Vice President of Science City Davos. He is representative to the AOTrauma R&D Commission from ARI.

Mauro Alini is Vice Director of the AO Research Institute Davos since 2009. He is an adjunct Professor at the Division of Orthopaedic Surgery of the McGill University, Montreal, Canada. He serves as a member of the Award Committee for The GRAMMER European Spine Journal Award. He is a Fellow of International Orthopaedic Research (FIOR) and a Fellow of the Tissue Engineering Regenerative Medicine Society (FTERM). He is co-Editor in Chief of the Journal Orthopaedic Research Spine. He is on the Assistant Editorial Board of the European Spine Journal. He is a member of the Scientific Editorial Board of the eCM Journal. He is also in the international Editorial Board of the Journal of Orthopaedic Translation and Journal Orthopaedic Research. He is representative to the AOSpine R&D Commission from ARI.

Boyko Gueorguiev–Rüegg is program leader of Biomedical Development at the ARI. He is an Honorary Professor at the Technical University of Varna, Bulgaria in the fields of biomedical engineering and biotechnology. He is Vice President of the European Orthopaedic Research Society (EORS). He is honorary Member of the Bulgarian Orthopedic and Traumatology Association and of the Serbian Trauma Association. He is a Member of the Academic Council at the University Multiprofile Hospital for Active Treatment and Emergency Medicine 'N I Pirogov', Bulgaria. He is appointed as Associate Editor and Editorial Board Member of the Journal of Orthopaedic Trauma and BMC Musculoskeletal Disorders, Section Editor for Orthopaedic Biomechanics at the Indian Journal of Orthopaedics, Academic Editor at the Editorial Board of Medicine, and Editorial Board Member of International Journal of Orthopaedics. He is representative to the AOTC System from ARI.

Martin Stoddart is a Principal Scientist and Program Leader for Regenerative Orthopaedics at the ARI. He is a full Professor at the Medical Faculty of Albert-Ludwigs University of Freiburg, Germany. He is honorary Professor at the Institute for Science and Technology in Medicine, University of Keele, UK. He is an elected Fellow of the Royal Society of Biology (FRSB). He lectures on the Skeletal Repair MSc module at the Department Health Science and Technology (D-HEST) of ETH Zurich. He is the Chair of the Orthopaedic Research Society (ORS) LearnORS Committee, and a member of the ORS Communications Council. He is Co-Deputy Chair of the International Cartilage Repair Society (ICRS) Basic Science Committee and an ICRS Fellow member. He is a member of the TERMIS EU Meeting and Sponsorship Committee. He is Scientific Editor for eCM Journal, an editor of BioMed Research International Orthopedics, an editor of Journal of Functional Morphology and Kinesiology, an Associate editor for Frontiers in Bioengineering and Biotechnology, and a member of the Review Editorial Board of Frontiers in Craniofacial Biology. He is the Co-coordinator and organizer of the yearly eCM conferences and a web editor of eCM Journal and eCM periodical. He is a member of the International Consortium for Regenerative Rehabilitation Leadership Council. He is the ARI representative to the AOCMF R&D commission.

Stephan Zeiter is a program manager of the Preclinical Services at the ARI. He is the past chair of the Preclinical Models Section of the Orthopaedic Research Society (ORS). He is a member of the scientific committee of the Swiss Laboratory Animal Science Association. For the European College of Laboratory Animal Medicine (ECLAM) he serves as a member of the council (treasurer) and is the president-elect. In Davos, he is committed as the vice president of the Society for Natural Sciences (NGD). Stephan is a member of the eCM International Review Panel and a guest lecturer in the MSc Course Skeletal Repair at the Department Health Science and Technology (D-HEST) of the ETH Zurich. He is the representative to the AOVET R&D Commission from ARI and the radiation safety officer.

Fintan Moriarty is a Principal Scientist and Focus Area Leader for Infection Biology at the ARI. He is a guest lecturer at the Bern University of Applied Sciences, MSc program in Medical Technology. Fintan Moriarty is a lecturer in the MSc Course Skeletal Repair at the Department Health Science and Technology (D-HEST) of the ETH Zurich. He is a scientific editor for the eCM Journal and a co-organizer of the annual eCM conference on the topic infection. He is also a member of the Editorial Board of Journal of Orthopaedic Trauma (JOT).

David Eglin is a Principal Scientist and Focus Area Leader for Polymers and Surfaces at the ARI. He is a council member of the Tissue Engineering and Regenerative Medicine International Society (TERMIS) EU Chapter. He is also a member of the International Editorial Board of Journal of Orthopaedic Translation (JOT). He is co-organizer of the annual eCM conference on the topic biofabrication. He lectures on the Skeletal Repair MSc module at the ETH Zurich and in the Biomedical Engineering MSc Program at the University of Bern. June 2019, David Eglin holds a professorship at the University of Twente, The Netherlands. In September 2020 Prof David Eglin took up a full-time position at the University of St. Étienne, France. We are sad to see him leave but wish him well and look forward to future collaborations!

Sibylle Grad is a Principal Scientist and Focus Area Leader for Disc and Cartilage Biology at the ARI. She is adjunct professor in biomedical engineering at the Department Health Sciences and Technology (D-HEST) of the ETH Zurich, organizer and lecturer of the ETH MSc Course Skeletal Repair and co-organizer of the course Practical Methods in Tissue Engineering. She is a member of the eCM Journal Editorial Board and a co-organizer of the annual eCM conference on the topic disc and cartilage. She is a member of the International Review Board of JOR Spine. Sibylle Grad is an EUROSPINE EduWeek Faculty member, ICRS Fellow member, and she is Vice president of the Graduate School Graubünden AG.

Peter Varga is a Focus Area Leader for Biomechanics and Modeling at the ARI. He is a lecturer of the virtual Tissue Biomechanics Laboratory course within the Master in Biomedical Engineering program at the University of Bern and currently completing his habilitation at the Medical Faculty. Peter Varga is a guest lecturer in the MSc Course Skeletal Repair at the Department Health Science and Technology (D-HEST) of the ETH Zurich. He is an academic editor of the BioMed Research International journal.

Other Professional Relations

Daniel Arens is member of the credential committee of Specialized Veterinarians in Laboratory Animal Science (SVLAS).

Angela Armiento was elected member of the ORS International Committee for a 3-year term (2018-2021). She is the ARI representative in the Program Committee of the Graduate School Graubünden AG. Since October 2020 she is the Project Manager for Italian in Zurich within the Native Scientist, a not-for-profit organization with the mission promote and exploit cultural and linguistic diversity in STEM through science outreach for children.

Valentina Basoli is lecturing at the University of Sassari Medical School, Italy on molecular biology, gene regulation and epigenetic within the course of biology.

Caroline Constant is a member of the Diversity Equity and Inclusion Committee of the American College of Veterinary Surgery (ACVS)

Elena Della Bella is Deputy Editor in Basic Science & Molecular Biology for Craniomaxillofacial Trauma & Reconstruction Open Journal (AO CMF journal). She is a member of tissue engineering and regenerative medicine society (TERMIS) and Orthopaedic Research Society (ORS).

Matteo D'Este was nominated Adjunct Professor at the Département de génie des mines, de la métallurgie et des matériaux of the Laval University, Québec City, Canada. He is a member of the Executive Committee of the Swiss Society for Biomaterials and Regenerative Medicine (SSB+RM). He is lecturer at the Department Health Science and Technology (D-HEST) of ETH Zurich, teaching Biomaterials for the Skeletal Repair and Advanced Hydrogels for the Practical methods in tissue engineering course. Matteo is member of the eCM Journal International Review Panel.

Yann Ladner is a member of the Young Scientists organizing committee of the Swiss Society for Biomaterials and Regenerative Medicine (SSBM+RM). He is also assistant for the Practical Methods in Tissue Engineering MSc course at the ETH in Zurich.

Zhen Li is a Visiting Professor at the Medical School of Shenzhen University, Shenzhen, China. She was elected as Member-at-large of ORS Spine Section Board for a 2-year term (2020-2021). She is the European Development Committee Member of International Chinese Musculoskeletal Research Society (ICMRS). Zhen Li is a member of the JOR Spine Advisory Review Board and eCM Journal International Review Panel.

Junxuan Ma was visiting scientist at the Science Foundation Ireland funded Centre for Research in Medical Devices (CÚRAM) at the National University of Ireland, Galway for 3 months.

Hansrudi Noser is an Adjunct Professor at the University of Zurich at the request of the Faculty of Economics. In addition, he acts as a member of the High School Graduation Committee of Liechtenstein. He retired in June 2020.

Marianna Peroglio is a certified Project Management Associate SGO. She was invited as visiting professor at INSA Lyon, France for 2 months. She is also a member of the eCM Journal International Review Panel. She left in September 2020 with her husband Prof David Eglin when he took up a full-time position in France. We are sad to see them leave but wish them well and look forward to future collaborations.

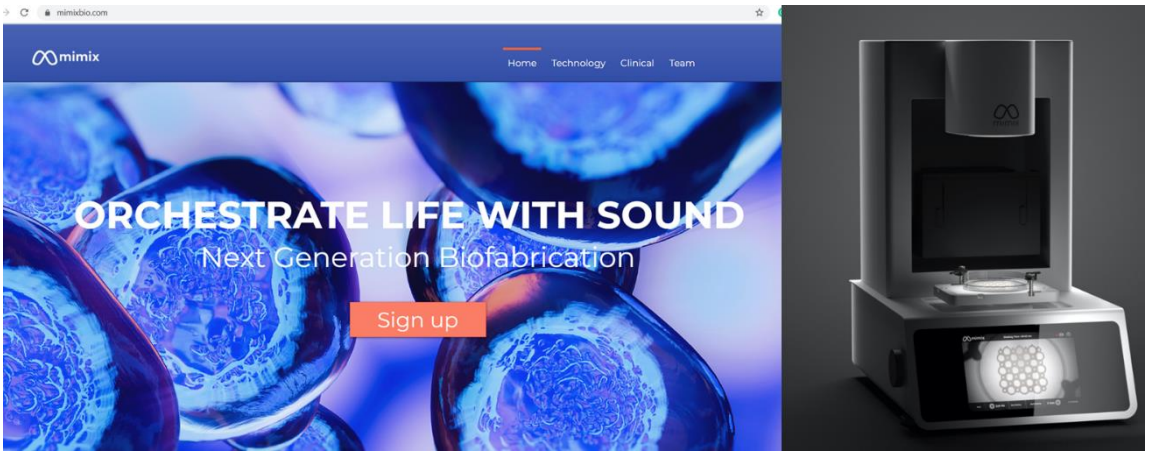
Christoph Sprecher is lecturer at the Fachhochschule Graubünden in Chur, at the block course for ETH/ZHAW students at ARI and for Tecday in Zuoz.

Sophie Verrier is a Principal Scientist at the ARI. She is board member and upcoming president of the Swiss Bone and Mineral Society (SBMS). She is also active member of the Orthopaedic Research Society (ORS) where she chaired the Women's Leadership Forum Committee and is member of the ORS Annual Meeting Committee. She is a member of tissue engineering and regenerative medicine society (TERMIS) and of the eCM International Review Panel (eCM Journal). She is also co-organizer of topic specific annual eCM conferences.

8 Good News

8.1 A new start up built on ARI Research IP

Mimix Biotherapeutics was incorporated in September 2019 with the appointment of Dr Tiziano Serra as Chief Scientific Officer. Appointment of Dr Mauro Alini and Prof Dr Geoff Richards in the scientific advisory Board. AO Foundation and mimiX enters a partnership agreement (MoU). mimiX is based on SIM technology previously developed at ARI. mimiX launched the first acoustic bioprinter, named CymatiX, on the market on Dec 15th, 2020.



mimiX webpage and the acoustic patterning device

Below are the main achievements of mimix in 2020.

2020

Feb

- SACHS Associates awards mimiX “Raising Stars”.

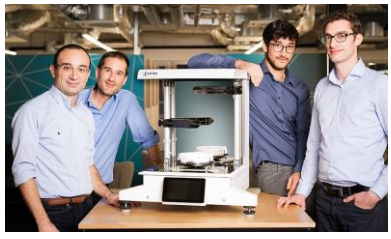
Mar

- mimiX is awarded with the VentureKick Phase 1.

Oct

- Publication of the Scientific work in the Biofabrication Journal (Sound-induced morphogenesis of multicellular systems for rapid orchestration of vascular networks).
- https://iopscience.iop.org/article/10.1088/1758-5090/abbb9c

- First prototype. Salsa3D is delivered and installed at ARI.



Aug

- mimiX is awarded with the VentureKick Phase 2.
- Dipl Ing Roman Amrein is appointed R&D Manager.

Sept

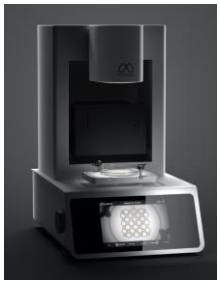
- Dr Dobrila Nesic is appointed Clinical Translation Manager.
- Granting of EUROSTAR Project to support Pont-Of-Care pre-clinical study. In partnership by Riginera HBW and AO Research Institute.

Nov

- InvestHorizon selects mimiX to be part of 80 European high-tech startups to enter the acceleratior programm for European institutional investments.
- New Corporate identity www.mimixbio.com

Dec

- Establishment and integration of the microcity incubator in Neuchâtel.
- Registration of CymatiX® product name.
- Support agreement of the Canton of Neuchâtel.
- mimiX Biotherapeutics unveils first acoustic bioprinter: *"Creating Life With Sound, the Next Generation of Biofabrication".* https://3dprint.com/276514/mimix-biotherapeutics-unveils-first-acoustic-bioprinter-creating-life-with-sound-the-next-generation-of-biofabrication/
- Top 10 Bioprinting Stories of 2020: Paving the Way to Future Organ Transplants. https://3dprint.com/276967/top-10-bioprinting-stories-of-2020-paving-the-way-to-future-organ-transplants/



8.2 New extramural funding

DePuy Synthes: 'Biomechanical comparison of different intramedullary nails for distal tibia fracture fixation'. Main applicant and coordinator of the project is Boyko Gueorguiev (ARI). Project funding is 139,000 CHF for 1 year.

DePuy Synthes: 'Biomechanical analysis of recently released cephalomedullary nails for trochanteric femoral fracture fixation'. Main applicant and coordinator of the project is Boyko Gueorguiev (ARI). Project funding is 112,000 CHF for 1 year.

University Hospital Basel: 'Biomechanical investigation of a new method for minimally invasive multidirectional stabilization of the acromioclavicular joint'. Project funding is 8,000 CHF for 1 year.

German Research Foundation (DFG), Special Research Area (Sonderforschungsbereich, SFB): 'Collaborative Research Centre 1313 – Interface-Driven Multi-Field Processes in Porous Media'. The project partners include Prof Boyko Gueorguiev (ARI), in collaboration with Professor Oliver Röhrle (University of Stuttgart). Overall 4-year project funding is 8.5 million Euro, ARI funding for project area 'Fluid-solid phase change' is 100,000 EUR.

Mereo BioPharma, UK: 'Multicentre placebo-controlled double-blind study in adult patients with type I, II or IV osteogenesis imperfecta treated with BPS804'. The aim of the project is to investigate the effect of a new anabolic drug on individuals with osteogenesis imperfecta including pre-clinical studies and a multicentre human clinical trial. The project partners include Peter Varga (ARI), McGill University, Canada, and University of Berne, Switzerland. ARI funding is 63,800 CAD.

Eurostars project OA-BIO: Life changing therapy for Osteo-Arthritis patients: a biomarker lead approach. The project partners include Zhen Li, Mauro Alini (ARI), in collaboration with Sonia Escaich (4Moving Biotech, France), Felix Eckstein (Chondrometrics GmbH, Germany), and Marianna Tryfonidou (Utrecht University, Netherland). Overall 3-year project funding is 1.5 million Euro, ARI funding is 250'000 Euro.

Eurostar project: E - RegenMed2.0, EU: "Re-Define Regenerative Medicine with a Point-Of-Care Tissue Production Technology".
The scope of the project is to validate a novel Point-Of-Care (POC) tissue production solution that will be at the core of a disruption in the field of personalized regenerative medicine. The solution aims to deliver a patient specific graft manufactured bed-side, starting with a small healthy biopsy of patient's own tissue, first converted into micrografts to be, in a second step, valorized as a transplant graft within a "Sound Induced Morphogenesis" biofabrication process. The project partners include Tiziano Serra, Mauro Alini (ARI), mimiX biotherapeutics (CH), RegeneraHB (IT). ARI funding is 150k CHF.

PREMUROSA: H2020 MSCA – ITN 2019 "Precision medicine for musculoskeletal regeneration, prosthetics, and active ageing".
Musculoskeletal diseases are a major burden on individuals, healthcare and welfare systems, and their treatment is currently based either on prosthetic or regenerative surgical procedures, often involving medical device implantation. The EU-funded PREMUROSA project aims to train a new generation of scientists with an integrated vision of the whole value chain in musculoskeletal regeneration technologies. The project will boost an environment of innovations that develop devices and optimised clinical applications using interdisciplinary, intersectoral and international approaches. The new generation of researchers will sharpen their experimental and complementary skills in a well-designed and diversified training programme. The project partners include Tiziano Serra, Mauro Alini (ARI). ARI funding is 300k CHF.

ON/EORS kick starter grant in Osteoarthritis research (20-149, 10 000€, 1 year) to investigate the plasticity of deep zone derived chondrocytes responding to environmental stimuli. Principal Investigator: Dr Andrea Schwab.

RISEus2 (M D'Este, M Alini) “Rising Competitiveness of Early Stage Researchers and Research Management in Latvia”. The aim of the RISEus2 project is to increase the research profile of early-stage researchers and strengthen the research management capacity of leading staff at RTU Rudolfs Cimdins Riga Biomaterials Innovation and Development Centre in the area of biomaterials development for bone tissue replacement and regeneration. The project is a close cooperation between the AO Research Institute Davos (ARI), Institut National Polytechnique de Toulouse CIRIMAT (INPT-CIRIMAT) and FORM-Lab Frankfurt Orofacial Regenerative Medicine, Goethe University Frankfurt (GUF). RISEus2 supported by European Union’s Horizon 2020 research and innovation programme (GA No 952347), has a duration of 3 years and a total budget of 900'000 Euros; ARI's budget is 143'000 CHF.

ImmunoBioInks EU Horizon 2020 programme (H2020-EU.1.3.2.) Individual Marie Curie Fellowship to Dr Jacek K. Wychowaniec. The project focusses on generating 3D printed constructs for treating musculoskeletal defects in immunocompromised patients (https://cordis.europa.eu/project/id/893099). Dr Wychowaniec will be joining the Biomedical Materials focus area to carry out this project. EU contribution is € 191,149.44.

3D Printed-Matrix Assisted Chemically Modified RNAs Bone Regenerative Therapy for Trauma and Osteoporotic Patients (cmRNA Bone) (M D'Este, M Stoddart) H2020-SC1-BHC-2018-2020. 11 partners, Total Budget €6.26 million, ARI Budget €710k, Period 2020-2023. The cmRNAbone project aims to create a novel bone regenerative therapeutic approach based on combination of chemically modified RNAs (cmRNAs)-vectors embedded in a 3D-printed guiding biomaterial ink tailored to patients need. Selected candidate formulations will be taken to clinically relevant preclinical proof of concepts. Finally, an overreaching effort on preparing a 1st in human trial will be taken, consisting of partner facilities auditing and clinical experts group support, to ensure that GMP-like production for all regenerative tools, and regulatory and commercial strategies are realized.

8.3 New AO Foundation intramural funding (grants beyond ARI retainer)

AO Strategy Fund (AOSF): 'OSApp: Virtual osteosynthesis tool for surgical education'. Main applicant and coordinator of the project is Peter Varga (ARI). Project funding is 522,000 CHF for 2.5 years.

AO Strategy Fund (AOSF): 'Digitally enhanced hands-on surgical training'. Main applicant and coordinator of the project is Jan Buschbaum (ARI). Project funding is 482,000 CHF for 3 years.

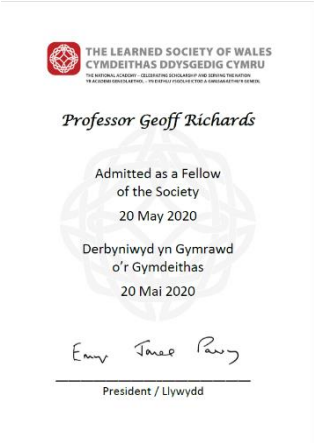
AO Development Incubator (AODI): 'Biphasic Plating – Next Generation Locked Plating'. Project partners are Markus Windolf (ARI) and Professor Devakar Epari (Queensland University of Technology). Overall project funding is 1.7 million CHF for 4 years.

AO Development Incubator (AODI): 'AO Fracture Monitor – Development Phase'. Main applicant and coordinator of the project is Markus Windolf (ARI). Overall funding is 4.0 million CHF for 4 years. The AO Fracture Monitor was created in ARI and is believed to be a major change to internal fracture fixation in the future.

8.4 Awards

Prof Geoff Richards elected Fellow of the Learned Society of Wales

The Learned Society of Wales is the national academy for arts and sciences.



Election to Fellowship is a public recognition of excellence. All our Fellows have made an outstanding contribution to the world of learning and have a demonstrable connection to Wales. The Society harnesses the expertise of the Fellowship with the explicit purpose of promoting greater awareness of how the sciences and the arts, humanities and social sciences benefit society. LSW have just under 600 Fellows, representing excellence in all branches of learning.
Fellow of the Learned Society of Wales (FLSW).

TERMIS-EU 2020 Mid Term Career Award

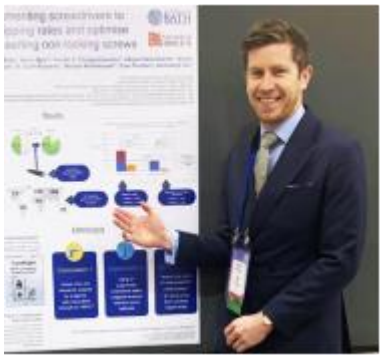
Prof Martin Stoddart was the recipient of the TERMIS EU 2020 Mid Term Career award. This prestigious award is in recognition of the substantial research contributions he and his team have made over the years. The Mid Term Career Award is for individuals that are within 10-20 years after obtaining their PhD. The Award recognizes an individual, who has demonstrated outstanding achievements in the field of tissue engineering and regenerative medicine research, during the midterm of his/her career with setting up a successful research group and clear evidence of outstanding performance.

Outstanding Poster Presentation

Alexandra Wallimann, PhD candidate at ARI in collaboration with SIAF, was awarded with the outstanding poster presentation entitled "The Influence of Microbial-Derived Metabolites on Bone Health, 18th EAACI Immunology Winter School, 23-26 January 2020, Chamonix, France.

ICORS Best Posters Award

James Fletcher was granted an ICORS Best Posters Award for presentation at ORS 2020. Title: 'TightRight: augmenting screwdrivers to reduce bone stripping rates and optimize tightness when inserting non-locking screws', in collaboration between ARI, Royal College of Surgeons, University of Bath and University of Bristol.



James Fletcher during his poster presentation at ORS 2020 in Phoenix, AZ, USA

Best presentation award at Graubünden Forscht for Gregor Miklosic and Stijn Rotman

Gregor Miklosic (bottom left) and Stijn Rotman (center left) being awarded for best oral presentation at the Graubünden Forscht Online Conference 2020.

The "Graubünden Forscht" conference has become a traditional appointment gathering scientists from our canton. The event shows the wide range and high level of research taking place in Graubünden. Like most conferences in 2020, Graubünden Forscht took place online, forcing the early-stage researchers to communicate their science in a new format. Gregor Miklosic and Stijn Rotman, PhD students in the Biomedical Materials focus area at ARI embraced this challenge and delivered outstanding online presentations which were awarded as best oral and poster presentations in the category Medical and Life sciences, together with Pattraporn Satitsuksanoa (Swiss Institute for Allergy and Asthma Research SIAF, Davos).

Bill Crawford award

Andrew Foster (2020 ARI MD Fellow) received the Bill Crawford award for best basic science registrar paper at the 2020 Annual Scientific meeting of the Australian Orthopaedic Association meeting on November 14, 2020 in Brisbane Australia.

8.5 ARI new MOU's (Memorandums of Understanding)

In June 2020, a new Memorandum of understanding for academic collaboration was signed between the Department of Oral and Maxillofacial Surgery, University Hospital Tübingen and AO Research Institute Davos. This builds of the collaboration between ex ARI Medical Fellow Dr med Andreas Naros and Prof Martin Stoddart as they continue to work on a joint AO CMF grant AOCMFS-19-07N.

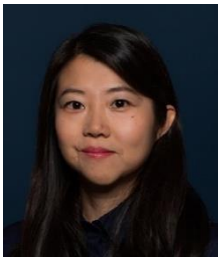
8.6 New Board Positions

European Orthopaedic Research Society (EORS)

Prof Boyko Gueorguiev, Biomedical Development program leader at ARI was elected to be Vice President in the executive committee (EC) of the European Orthopaedic Research Society (EORS). Following his previous function as General Secretary, he was elected at the Society's General Assembly held at the virtual EORS 2020. This position continues keeping a strong connection of the AO Foundation with the EORS.

Prof Boyko Gueorguiev moderating the EORS 2020 General Assembly.

Dr Matteo D'Este, Biomedical Materials Focus area leader a.i. was nominated Adjunct Professor at the Département de génie des mines, de la métallurgie et des matériaux of the Laval University, Québec City, Canada. This is a wonderful achievement and highlights the internationally recognized work performed at ARI.



Dr Zhen Li, Senior Scientist in the Regenerative Orthopaedics Program, was elected as Member-at-large of ORS Spine Section Board for a 2-year term (2020-2021).

Dr med vet Stephan Zeiter (ARI) and Katja Bärenfaller (SIAF and Science city Davos) were elected as co-presidents of NGD (Naturforschende Gesellschaft Davos).

8.7 Habilitation

Major career achievement for ex-ARI Fellow

ARI Fellow Dr Gernot Lang, currently a resident surgeon at the department of Trauma and Orthopedic Surgery, University Medical Center Freiburg, Germany, successfully defended his Habilitation from the University's medical faculty. The focus of his habilitation thesis was on "Staged Therapeutic Concepts in Degenerative Disc Disease Including Biological and Minimally Invasive Treatment Approaches". We congratulate Gernot to this major achievement in his professional career which awards him the "Venia Legendi" at the Medical Faculty in Freiburg.

Gernot Lang joined the ARI's Regenerative Orthopaedics program in 2014 for a 12-month medical research fellowship. He was involved in several ARI projects related to intervertebral disc research and substantially contributed to the outcome of the EU-FP7 funded project entitled "Biomimetic Nano-Fiber-Based Nucleus Pulposus Regeneration for the Treatment of Degenerative Disc Disease".

Left: Prof Norbert Südkamp Right: Dr Gernot Lang

After another postdoctoral Spine Research Fellowship at Weill Cornell Medical College, Department of Neurosurgery, New York Presbyterian Hospital, he continued his residency at the department of surgery of the University Medical Center Freiburg.
Since then, Gernot and ARI have established an ongoing collaboration that has been strengthened by successful grant proposals funded by different agencies including the SET Foundation for the promotion of alternate and complementary methods to reduce animal testing in research, the German Spine Society, and the German Arthritis Foundation.
This fruitful collaboration has led to the co-supervision of to date six MD candidates affiliated at the University of Freiburg who have carried out their practical theses within the ARI laboratories. ARI's co-investigators of these collaborative projects are Zhen Li, Sibylle Grad and Mauro Alini.
The remarkable output comprises six co-authored publications, many congress contributions and further successful grant applications. This demonstrates the interactions between the ARI Regenerative Orthopaedics team and the Experimental Spine Research Group of UMC Freiburg will continue.

ARI Fellow Mario Morgenstern successfully defended his habilitation at the University Basel and presented his research work in his inaugural lecture entitled “Fracture-related infections – from the beginning of the AO up to most recent treatment concepts” in August 2020. Mario Morgenstern was an ARI Fellow in 2012 in the Infection Biology Group headed by Dr Fintan Moriarty. His research focused on clinical and experimental aspects of bone and joint infections. During his residency at the Trauma Center Murnau in southern Germany (Berufsgenossenschaftliche Unfallklinik Murnau) he established a close research collaboration with the ARI, resulting in three further MD fellows from Murnau succeeded him in ARI. Within the AO Clinical Priority Program Bone Infection (lead by Prof SL Kates and Prof E Schwarz) Mario Morgenstern contributed significantly to the world-wide bone infection registry as principle investigator at the site Murnau and to various other projects within the research program.
Since 2016 he is working as an orthopaedic surgeon at the Department of Orthopaedic Surgery and Traumatology at the University Hospital Basel, only interrupted by a clinical research fellowship at the Oxford Bone Infection Unit under the mentorship of Martin McNally (former president of the European Bone and Joint Infection Society).
Mario Morgenstern, and his colleague and friend Willem-Jan Metsemakers (UZ Leuven, Belgium), initiated the Fracture-related Infection (FRI) Consensus Group that was supported by the ARI and AO Foundation and the European Bone and Joint Infection Society. In a first consensus meeting in Davos in 2016, a consensus definition for FRIs was established, which was later accepted as working definition at the 2018 International Consensus Meeting (ICM) on Musculoskeletal Infection. After a second meeting of the Fracture-related Infection (FRI) Consensus Group in 2018, the definition was updated and recommendations for diagnosis and treatment of FRIs were established and published.
Mario Morgenstern is currently working together with Martin Clauss and Parham Sendi at the recently founded interdisciplinary Center for Musculoskeletal Infections at the University Hospital Basel and is heading the clinical research unit at the Department of Orthopaedic Surgery and Traumatology. The Center for Musculoskeletal Infections at the University Hospital Basel and the Infection Biology Group at ARI are continuing a close research collaboration with ongoing projects. Both groups will be the joined organizers of the Annual meeting of the European Bone and Joint Infection Society in 2022 in Basel.
Mario Morgenstern published 50 Pubmed listed publications and 3 book chapters (including a chapter in the AO Infection Manual) mainly on bone and joint infections.

8.8 Collaborations

AO Research Institute Davos implements remote meeting platforms to share best research practices with the Baltic Biomaterials Center of Excellence

The EU project BBCE, Baltic Biomaterials Center of Excellence, officially started on January 1st, 2020. This project, mostly based on bilateral mobility of researchers to gain knowledge about best practices in biomaterials and musculoskeletal research, was obviously subjected to major repercussions from the global ban on travel. With the goal of implementing at least part of the objectives set for the project, the ARI team in collaboration with BBCE selected the topics most suitable for implementation in a virtual format and established a calendar of 18 virtual meetings held between end of July and beginning of September 2020. Thanks to a great effort from all speakers involved, the teaching content, which was initially planned to be delivered by working side-by-side with the guests, was transferred into a virtual format. With excellent support from the AO IT department and after a few pilot tests with the mobile videoconferencing systems, this series of meetings turned into a success, as measured by the feedback forms filled in by the participants after each virtual visit.
The topics covered in the series were: 3D image-based planning and analysis; Implementation of Quality Systems according to ISO and GLP standards in BBCE core institutions to enhance performance and reputation, with specific training on ISO 9001, ISO 13485, and GLP/GMP; laboratory management, cell culture databases; brief history of eCM journal and conferences; conference organization for networking; journal citations, impact factor, h-index, open archive; histology laboratory management, histology databases, data management for industry projects; websites and social media; diversity, inclusion and mentorships initiatives at AO; research ethics; experimental planning; electronic lab journals. The average number of participants per visit ranged between 26 and 45. Thus, one benefit arising from the online format was the opportunity to extend the invitations beyond the limited number of participants who would have travelled; additionally, the invitation was extended to the whole ARI. Overall, the experience was very positive for all the institutions involved

Figure 8.8.1. Local (top) and virtual (bottom) audience during the virtual meeting on the history of the eCM journal and conferences, held on August 26th, 2020.

Partners:
AO Research Institute Davos
Institute of Biomaterials at the Department of Materials Science and Engineering of the University of Erlangen-Nuremberg

Core BBCE partners:
RigaTechnical University Rudolfs Cimdins Riga Biomaterials innovations and development centre (RTU RBIDC)
Latvian Institute of Organic Synthesis
Riga Stradins University
Riga Stradins University Institute of Stomatology.

9 AO Research Institute Davos Medical Research Fellows

The ARI's Research Fellowship program again attracted resident and senior surgeons from around the world. Some of the many benefits to a surgeon are:

- Creation of tangible results in research
- Possibility of medical publication as a co-author (depending upon time and input)
- Knowledge on how to approach research challenges in future
- Inspiration from being part of a world renowned international multidisciplinary R&D team
- Inside knowledge attainment of the AO Foundation
- Enlarging personal networks for future R&D and AO Foundation activities
- Chance to have a research friend/mentor that is always easy to contact

ARI Fellows, 2020

Research Fellows

Susanne Baertl: University Medical Center Regensburg, Department of Trauma Surgery, Regensburg, Germany
ARI Project: **Comparison of a high-virulent versus a low-virulent *Staphylococcus aureus* strain in a murine bone infection model**
During my six-month stay at the ARI, I had the chance to join the Focus Area on Infection Biology as a medical fellow. In collaboration with the clinic in Regensburg, we focused on a mouse model for fracture-related infections to compare two different clinically isolated *Staphylococcus aureus* strains in terms of clinical presentation, systemic inflammation, bacteriology, and histopathological evaluation. Later on, we extended the project to an *in vitro* part, highlighting differences in iron acquisition ability between these two strains. This project was a great opportunity to get in touch with international experts from different fields and gain knowledge in various disciplines from renowned scientists.
Another highlight during my fellowship was joining the first completely virtual AO course, meeting surgeons and participants from all over the world. Last but not least, I really appreciated the warm and friendly atmosphere at the ARI, where it was possible to find friends for life. Being at this unique place of orthopedic innovation in the middle of the mountains was an outstanding experience that I do not want to miss.

Lena Gens: University of Veterinary Medicine, Hannover, Germany
ARI Project: **Surgical technique and comparison of autologous and allogenic cancellous bone grafts from various donor sites in rats**
During my stay at ARI as a veterinary research fellow in the Preclinical Facility I supported the team in all running animal studies and gained an insight in the field of preclinical research and laboratory animal medicine. I started working on my doctoral thesis, my first time of doing research on my own. The whole team at ARI, whether at the PCF or the other groups, consists of so many specialists that are happy to share their knowledge and it was a pleasure working with all of them and having their support. I am very happy that I got the possibility to stay for three more years at ARI to do a residency in laboratory animal medicine and I am looking forward to continuing working in this international research atmosphere and to experience ARI and Davos in a time after Corona restrictions.

Walker Magrath: Johns Hopkins School of Medicine, Baltimore, USA
ARI Project: **1. Osteotomy PEEK plate murine model for studying the effect of SCFAs and antibiotics on bone regeneration. 2. The effect of SCFAs and the probiotic EPS on chondrocyte differentiation. 3. Literature review of the impact of SCFAs on bone healing.**
During my time in Davos as a medical research fellow in Infection Biology, I spent most of my time researching the effect of microbiota-derived metabolites, short chain fatty acids (SCFAs), on bone regeneration. We collaborated with ARI's Preclinical Facility to design and implement our mouse model and worked with other Programs for subsequent biomechanical testing, *in vitro* experiments, and microbiological assays. Having the chance to work at ARI so early into my medical career gave me the unique opportunity to meet scientists and clinicians from all over the world and underscored the value of clinically inspired translational research. My first time living in Europe, I ran countless miles in the mountains, enjoyed après ski with friends, and traveled across Europe. I met many wonderful people in Davos, ready to welcome me. No other place can you work with world renowned surgeons and scientists and ski beautiful Alpine pistes in the same day!

Torsten Pastor: Cantonal Hospital, Luzern, Clinic for Trauma- and Orthopaedic Surgery, Lucerne, Switzerland
ARI Projects: **1. Biomechanical analysis of recently released cephalomedullary nails for trochanteric femoral fracture fixation in a human cadaveric model. 2. Helical dual plating of complex distal femur fracutes. 3. Virtual reduction of humerus fractures.**
During my stay at ARI as a medical research fellow in the Biomedical Development Program I mainly focused on studies about different implants. Especially the multidisciplinary approach to solve problems fascinated me. Moreover, a great infrastructure for research in an international team really motivated me to begin new projects. I highly recommend working within the ARI and living in Davos with its stunning surroundings.

Hella Schwegler: University of Veterinary Medicine, Hannover, Germany
During my stay at the Preclinical Facility at ARI I had the chance to go along different projects. I got insights into planning and setting up a study plan; throughout the research process I took part in the initial surgeries and revisions, postoperative care, and medications to the end of the project with the euthanasia of the animals and the evaluation of the results. My fellowship at ARI was very practical with lots of hands-on experience, responsibility, and a great, dedicated team, that shared their knowledge with me and made the workdays real fun. I felt welcome at the ARI from the very first day, my work colleagues became good friends, and I enjoyed the beautiful surrounding in Davos, especially the empty ski slopes during the week. Thanks for this wonderful experience.

Mai Thanh Vo: University of Veterinary Medicine, Hannover, Germany
During my stay at ARI as a vet fellow in the preclinical facility I was involved in various animal studies. Moreover, I could acquire further experiences and knowledge in the field of preclinical research. I was able to participate in diverse animal studies and investigate almost every aspect of this field of work. I am grateful to have the opportunity to be part of a team consisting international and renowned scientist. Therefore, I had the chance to broaden my horizon and expand my scientific knowledge as well as my skillset as a veterinarian. During my stay in Davos, I made many wonderful memories.

Hao Wei: Shandong Provincial Third Hospital affiliated to Shandong University, Department of joint and sports medicine, China
ARI Project: **Optimizing osteogenic protocol for in-vitro induction of mesenchymal stem cells**
During my stay at ARI as a medical research fellow in the Regenerative Orthopaedics Program, I focused on the interaction between the human body and orthopaedic implants with different specific surface properties. Specifically, I tried to help improve the existing osteogenic protocol for the in-vitro induction of mesenchymal stem cells (MSCs) in order to simulate more precisely their behavior *in vivo*. The time at ARI was full of new, exciting experiences. Working within an experienced international team highly broadened my knowledge in basic research. ARI provided an ideal and unbelievable infrastructure for research at the top level all over the world. The enthusiasm, innovation and kindness among group members deeply affected me and inspired me a lot. The mountains and surrounding valleys also have my footprints left. It is definitely an unforgettable experience during my whole life. I will highly recommend my colleagues to work here within ARI.

Katie Young: University of Bristol, UK
ARI Project: **Local delivery system for improving efficacy of bacteriophages combined with antibiotics**
The AO Research Institute is a melting pot of medical and technical expertise. There are engineers, microbiologists and biomaterial scientists among others – any question you have, you only need to take a flight of stairs. The technical facilities are incredible. I very much appreciated the non-hierarchical structure and the friendly atmosphere at the ARI. Everybody's ideas are welcome and are always accepted in a receptive atmosphere. During my time as an ARI Fellow, I worked in Dr Fintan Moriarty's Focus Area on Bone Infection and was fortunate to have been part of an international team which, in a joint project with Dr Andrej Trampuz of the Center for Musculoskeletal Surgery at Berlin's Charité hospital and Prof Dr Willem-Jan Metsemakers of the Department of Trauma Surgery, University Hospitals Leuven, in Belgium, is trialling a new technique for the treatment of bone infections. The approach involves incorporating both antibiotics and bacteriophages – viruses, which exclusively infect bacterial cells – in a hydrogel, which can then be applied directly to an infected bone in order to eradicate the responsible pathogens.
Having twice had the chance to benefit from AO Foundation research grants during my surgical training in the UK, it was a real honor to be able to come to Davos in person for this fellowship. I really enjoyed being able to look at the medical world from a fresh angle and to see how scientific research informs medicine and healthcare. The opportunity to contribute to the organization and running of some of the renowned AO courses was an added-on bonus of my time there. The ARI is in the middle of a beautiful valley in the Swiss alps. I am a keen runner and skier, and during my fellowship I very much made the most of this magical location.

Guest Students

Teresa Brose: Albert-Ludwigs University, Freiburg, Germany
ARI Project: **Identifying novel therapeutic targets for articular cartilage repair**
I am a medical student at the Albert-Ludwigs University in Freiburg (Germany) and was at the ARI for my doctoral thesis. I joined the Regenerative Orthopaedics program and worked on a project where we co-cultured human mesenchymal stromal cells and chondrocytes to study their regenerative potential under mechanical loading. I am very appreciative of the unique opportunities to experience translational research first-hand, learn and be trained by exceptional scientist, and all that while being met on equal footing. Its geographical location completes the list of great features, making my stay in Davos unrivalled.

He Chang: University of Veterinary Medicine, Vienna, Austria
ARI Project: **A humanized mouse model for investigating *Staphylococcus aureus* implant-associated infections**
I am a veterinary graduate from the University of Veterinary Medicine Vienna, Austria. I joined the preclinical facility of the AO Research Institute Davos as an intern, where I later rejoined as a fellow. During my stay at ARI, I have been closely involved in various projects regarding orthopedic infection research using animal models. One of my main responsibilities was to provide state-of-the-art perioperative care for the animals. My experience from meeting such a great variety of wonderful people I have been working with and from which I learned of can only be summed up as memorable. Needless to say, Davos is gorgeous to say the least.

Chen Guoliang: Sun Yat-sen University, Guangzhou, China
ARI Project: **Dual fluorescence functional mesenchymal stem cell sorting**
I am a medical doctor in spine surgery and currently doing my PhD research at Sun Yat-sen University. In the past few years, I majored in the clinical research of spine surgery and the basic research about the treatment of spinal cord injury with stem cells. During the period of 12 months at AO Research Institute Davos, I focused on the dual fluorescence functional mesenchymal stem cell sorting. I come from the southern China; a region that never has snow. In Davos these days, I have learned skiing and enjoyed the beauty of mountains by ski touring. The journey at AO Research Institute Davos will be a wonderful memory for me.

Manuel Herzog: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **miRNA analysis to discover fracture related biomarkers**
I am a master's student in molecular health sciences and currently writing my Master's thesis about miRNAs that are involved in the mechanically driven differentiation process of MSCs. This project allows me to connect my broad knowledge in the field of health sciences obtained during my studies at ETH Zurich with firsthand lab work here at ARI. Besides, Davos is a great place to work since I am a passionate skier. The beautiful mountains provide one with the ideal balance to the demanding and interesting work.

Takuya Hidaka: Kyoto University, Japan
ARI Project: **Optimized chondrogenesis in an osteochondral defect model**
I am a PhD student and doing my PhD study at Kyoto University in Japan. I work on a chemical biology research to develop DNA binding molecules which bind to a specific DNA sequence and modulate cellular function. To apply our compounds to the control of mitochondrial and bacterial DNA transcription, I worked with the progenitor cell biology group and the infection biology group for two months. It was a great opportunity for me to learn new experimental skills and multidisciplinary knowledge with highly motivated members in ARI. During my stay, I enjoyed walking around Davos surrounded by beautiful mountains and took many pictures, which are wonderful memories for me.

Edera Marcello: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **An enzybiotic-based approach for the treatment of *S. aureus* fracture-related infections**
I am a Food Scientist student, that has recently graduated with a bachelor's degree. As part of my bachelor's thesis, I had the opportunity to be part of the Enzybiotics project at the AO Research Institute in Davos. We investigated an enzyme-based approach to treat *S. aureus* fracture-related infections, conducting an in-vivo animal study of femur fracture as well as two in-vitro studies. I had such an awesome time at the ARI, gaining a lot of lab experience whilst also greatly expanding my knowledge. One thing that inspired me about the ARI was the amount of knowledge and expertise that I encountered there. Scientists from different areas connect and constantly strive for innovative alternatives to solve current orthopedic issues. Besides the great work experience, I was able to enjoy the mountains and make new friends that made me feel very comfortable and welcomed.

Guo Peng: Sun Yat-sen University, Guangzhou, China
ARI Project: **Cartilage repair with biomimetic decellularized extracellular matrix biomaterials**
I am a PhD candidate majoring in spine surgery at Sun Yat-sen University. At the AO Research Institute Davos, I studied as a visiting PhD student member in the Regenerative Orthopaedics group from Dec 2020 to Dec 2021 where I had the precious opportunity to learn a lot on decellularized extracellular matrix biomaterial preparation and application for cartilage repair. Davos is a skiing paradise and I have learned this exciting new sport which was an extraordinary experience in my whole life.

Babak Saravi: University Medical Center, Freiburg, Germany
I am an MD/DMD research scientist at the University of Freiburg. As a member of the Regenerative Orthopedics program, I focused on the so-called tissue renin-angiotensin system's role and its impact on degenerative disc disease progression. The experience in ARI was an essential part of my curriculum. It strengthened my scientific knowledge and lab skills in a multidisciplinary, internationally renowned scientific environment. Regular meetings and feedback and the involvement in the project planning were part of this process and helped me to improve my way of working. As an additional bonus, I could conduct this learning process in the beautiful mountains of Switzerland. I am pleased to be part of the ARI family.

Maja Strunk: University of Veterinary Medicine Hannover, Germany
I am a veterinary medicine student and will finish my study in April 2021. I had the opportunity to spend two months in the preclinical facility at the AO Research Institute Davos in July and August 2020. The veterinarians there are performing studies in animals before the clinical stage is reached. There I worked with the veterinarians in surgery, anesthesia, and medical care. I got the chance to get a really good impression of human medicine research work in animal models. Since a lot of projects were running when I arrived in Davos, I did not work in one special project, but I joined the running ones and supported the daily work at the PCF. I spent an incredible summer in the mountains, made some new friends from different places all over the world and set the first stone in my research carrier. For sure I will come back some time.

Penghui Zhang: Sun Yat-sen University, Shenzhen, China
ARI Project: **Anti-inflammatory therapy for cartilage preservation**
I am a PhD candidate in orthopedic surgery at Sun Yat-sen University and focusing on bone and cartilage regeneration field. I was proud of being a member of cartilage regeneration group. As a medical fellow, I gained an opportunity to study the anti-inflammatory and regenerative effect of small molecules for cartilage preservation with *in vitro* and *ex vivo* models. During the period of one year at AO Research Institute Davos, I acquired comprehensive working expertise in cell biology, tissue explant culture, molecular biology, and histology techniques due to instructions from my tutors and line manager. Additionally, the beautiful snow and exciting skiing sport in Davos impressed me.

Internships

Alig Gion: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **3D bioprinting of intervertebral tissue analogues**
I am currently doing my Master's degree in Biomedical Engineering with a focus on Molecular Bioengineering at ETH Zurich. I joined the Regenerative Orthopaedics program at the AO Research Institute Davos for my 3-month internship in the field of intervertebral disc regeneration/engineering. For my subsequent Master's thesis I focused on freeform 3D printing. I really enjoyed working at ARI and gained many experiences in the practical laboratory work.

Antonacci Paolo: Polytechnic of Turin, Italy
ARI Project: **Cartilage and subchondral bone visualization and quantification with contrast enhanced computed tomography**
I am currently finishing my master's degree in Biomedical Engineering at the Politecnico di Torino. As part of my master thesis with related traineeship, I joined the Medical Imaging group of the Biomedical Department. I focused on the automatic segmentation of condyles, automatic generation of volume of interest and images registration. It was really a pleasure working in a very professional and welcoming team and I improved my research skills in the fields of Medical Imaging. Moreover, I loved the opportunity to combine the scientific work with my passion for mountain sports.

Amirsiavosh Bashardoust: EPFL Lausanne, Switzerland (Country Iran)
ARI Project: **Statistical analysis of complex proximal humerus fractures**
As a Master's degree student in Mechanical Engineering with a specialization in biomechanics, it seemed only natural to me to come to the AO Research Institute Davos (ARI) for my mandatory six-month internship. I spent my placement in the Biomedical Development program, where I examined how the digital processing of images from CT scanners could be combined with statistical analysis in order to enhance the fixation of bone fractures.
Originally from the Iranian capital Tehran, I came to Switzerland to attain my Master's degree at the at the École Polytechnique Fédérale de Lausanne (EPFL) in the French-speaking Romandie. That experience, coupled with the opportunity to spend time in Davos in the German-speaking part of the country, has considerably broadened my horizon, both on a personal and a professional level.

Janick Eglauf: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **Link mechanics, degeneration and discogenic pain**
I am currently studying Health Sciences and Technology with a major in Medical Technology. I joined ARI for an Internship and my Master's Thesis and was part of the sound guided tissue regeneration group and the disc and cartilage group. I focused on the link between IVD degeneration and disc nerve ingrowth and developing a 3D organoid-like co-culturing model to investigate the influence of AF tissue on DRG outgrowth. Due to the work in such an international and interdisciplinary team I gained practical experiences in research and was able improve my knowledge in tissue engineering and disc degeneration. The location of ARI in the swiss alps enabled the combination of research and my passion for skiing.

Yanan Fu: Beijing University of Chemical Technology, Beijing, China
ARI Project: **A nanoparticle-based approach to target and evaluate intracellular *S. aureus* "Trojan horse" macrophages in fracture-related infection**
I am currently completing my Doctor's degree in Chemical Engineering and Technology with a focus on Biomedical Materials at Beijing University of Chemical Technology. As part of my fellowship and doctor thesis, I had the opportunity to do a 6-month internship in infection group. I focused on nanoparticle-based approach to target intracellular pathogens. I was excited to work together with an interdisciplinary team of international scientists and got an insight in professional scientific work in the fields of fracture related infections. Besides this, I loved the opportunity to combine the scientific work with my passion for skiing.

Joseph Hintermann: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **Osteogenic differentiation of MSCs with antibiotics co-cultured with *S. aureus in vitro***
I joined the Infection Biology group for my internship and master thesis to complete my master's degree in Health Science and Technology, majoring in Medical Technology at ETH Zurich. My focus was on finding therapeutic concentrations of antibiotics for local application while preventing infection and enabling bone regeneration. The combination of living in a small, Swiss city surrounded by mountains while working with an international team stemming from various backgrounds was refreshing and interesting. Learning from engineers, microbiologists and biomaterial scientists all at the same time and at

the same place while improving my research skills was an unforgettable experience. And being able to go skiing or hiking with friends at short notice is definitely underrated.

Thomas Jörimann: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **Percentage of compressive strain inducing hypertrophic mesenchymal stem cell differentiation *in vitro***
I am currently finishing my master's degree in Health Sciences and Technology with a major in Medical Technology at the ETH Zurich. I joined the AO Research Institute Davos to do a 3-month internship and my master thesis. My aim in the Regenerative Orthopaedics group was to investigate the effect of mechanical stimulation on controlling the mesenchymal stromal cell differentiation fate. I was excited to gain experiences with the methods of Tissue Engineering and working in a multidisciplinary research environment, all in combination with my passion for skiing and mountains.

Manuel Knecht: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **Development of an interactive osteosynthesis learning platform**
I joined ARI after having finished my bachelor's degree in Mechanical Engineering at the ETH Zurich. Here I worked in the Biomechanics and Modelling Group on the OSapp project and focused on the validation of the simulation results and helped to develop and program the learning platform. This internship at ARI gave me the possibility to get to know the field of biomechanics and biomedical development and to make use of my engineering knowledge on an interesting and fascinating subject. I enjoyed working in a friendly and supportive environment and contributing to an innovative research project. I loved the cold, white winter in Davos, making new friends and spending time out in the nature to go hiking or skiing.

Andrea Nüesch: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **The influence of mesenchymal stromal cells secretome on nucleus pulposus cells exposed to a proinflammatory environment**
Currently I am finishing my master's degree in Health Sciences and Technology at ETH Zurich. My major is in medical technology with the focus on tissue engineering. I joined the disc regeneration group at ARI as part of my fellowship and master thesis for 10 months. The focus of my stay was studying the effect of the secretome of mesenchymal stem cells on the regeneration of intervertebral disc cells. At ARI I could improve my research skills and learned how to work in an interdisciplinary environment. The location of the research institute in the middle of the mountains was pure joy for me as a mountain lover.

Lotta Reimann: University of Veterinary Medicine, Hannover, Germany
The two-month internship was part of the practical year of my study in veterinary medicine. I had the opportunity to get insights into ongoing projects of preclinical research. The diversity resulted from working with different animal species, multiple surgical techniques and versatile diagnostic monitoring, among other things. This variety was complemented by an instructive collaboration with an open-minded interdisciplinary team. I used the snowy time in Davos for my first skiing experiences and long hiking tours through nature. Moreover, I had the opportunity to travel and explore other Swiss cities.

Magdalena Remppis: Stuttgart University, Stuttgart, Germany
ARI Project: **Predicting patient-specific mechanical failure of proximal humerus fracture plating with computer simulations**
I am studying Biomedical Engineering, with a focus on Biomechanics, in the master's program at the University of Stuttgart. For my master thesis, I got the opportunity to join the Biomedical Development Program at ARI in Davos. My research contributes to shoulder activity tracking, where I am processing and analyzing activity data from accelerometers taken from a clinical study to investigating post-operative patients' movement. Working on this project helped me improve my research skills and enabled me to collaborate in a fresh-minded interdisciplinary team contributing to state-of-the-art research. In my spare time, I enjoyed hiking and skiing in the snowy landscapes of Davos.

Flurina Staubli: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **Multiple crosslinked bio-inks for 3D microextrusion of tissue-like constructs**
I recently finished my master's degree in Biomedical Engineering with a focus on Molecular Bioengineering at ETH Zurich. To write my master's thesis, I joined the Biomedical Materials group and worked on the 3D bioprinting of stem cell spheroids in hyaluronic acid and collagen I hydrogels. By working on this multidisciplinary topic, I was able to gain experience in lots of different laboratory methods and learned a lot about tissue engineering and regenerative medicine in general. Outside of work I spent most of my time enjoying the mountains by either hiking, climbing, or snowboarding.

Win-Hon Trinh: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **Biomaterials as delivery system of bacteriophages for the treatment and prevention of infections**
I was in my last semester in Health Sciences and Technology with a major in Medical Technology at the ETH Zurich. As part of my fellowship and master thesis, I joined the Biomedical Materials and Infection group. The aim of my project was to compare various biomaterials as carriers for bacteriophages in the treatment and prevention of biofilms caused by Staphylococcus aureus and Pseudomonas aeruginosa. I enjoyed working in an interdisciplinary team, combining the field of biomedical materials with infection treatment. Furthermore, I enjoyed having the mountains right in front of my house, ideal for skiing on a weekly basis.

Sylvie Wirth: Swiss Federal Institute of Technology, Zurich, Switzerland
ARI Project: **Bottom Up Printing Approach**
I am a Master Student in Health Sciences and Technology at the ETH Zurich focusing on medical technology. During my 3-months internship followed by my master Master's Thesis at the ARI I was working on the development of a 3D printed LEGO scaffold for mandibular defects. I was happy to gain some hands-on experience in a laboratory environment and to contribute to the project. Further, I very much appreciated the exchange with other researchers and enjoyed my time in beautiful Davos.

10 Project Abstracts by Sponsors

10.1 AOCMF

Bottom up printing approach (BUPA2) (Started) (A Armiento, M Stoddart)

Background: 3D-printed personalised scaffolds are an attractive approach for mandibular bone repair. The challenging loading environment of this site requires biomaterials with suitable mechanical resilience, which may be provided via the addition of flexible materials such as thermoplastic polyurethane (TPU).
Goal: This work aims to create a 3D printable personalised scaffold with a configurable layered composition, enhanced mechanical properties and improved cell adhesion.
Results: Varying material combinations are mixed to obtain a printable ink (RegenHu Discovery®). After printing, surface microporosity and cytotoxicity is assessed using scanning electron microscopy (SEM) and CellTiter-Blue®, respectively (Figure 10.1.1A). A 3D model of a mandibular defect is derived from CT scans, then sliced and modified with CAD to obtain LEGO®-like structures. The personalised scaffolds are printed as a series of layers incorporating an interlocking mechanism (Figure 10.1.1B). Scaffolds with precise and interconnected filaments can be printed and SEM images show surface microporosity, while no cytotoxicity is reported in 3T3 cells. Large scale personalised mandibular implants can be successfully printed and assembled.

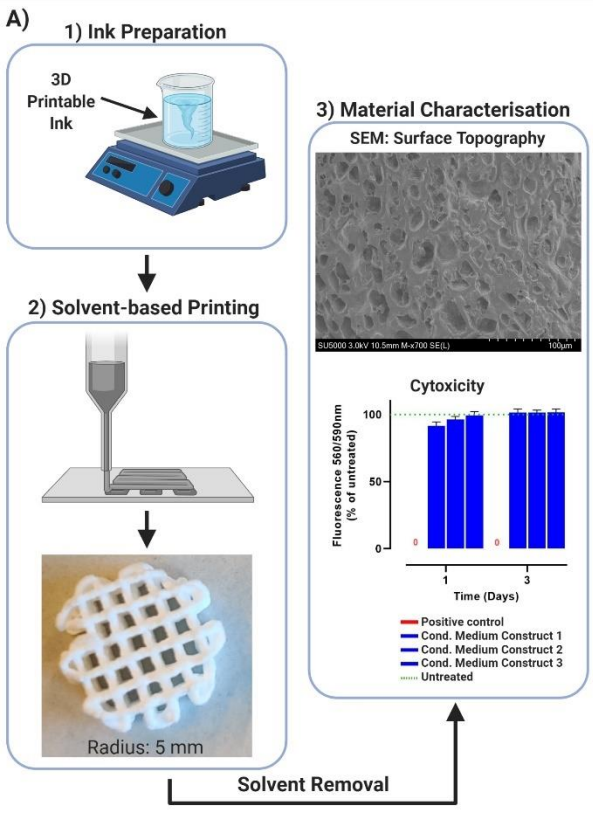


Figure 10.1.1A

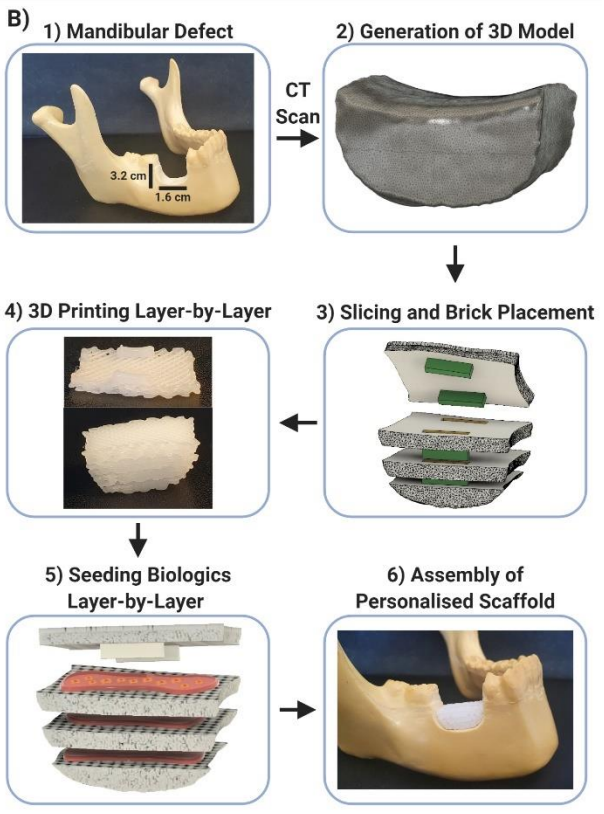


Figure 10.1.1B

Pub:
Johannes Hasler, Luan Phelipe Hatt, Martin James Stoddart, Angela Rita Armiento. Stable Reference Genes for qPCR Analysis in BM-MSCs Undergoing Osteogenic Differentiation within 3D Hyaluronan-Based Hydrogels. *Int. J. Mol. Sci. 2020, 21(23), 9195.*

Partner:
- Zenobi-Wong M (Prof), Institute for Biomechanics, ETH Zurich, Switzerland

Investigating effects of BMPER on osteogenic and chondrogenic differentiation (Started) (R Rothweiler, M Stoddart, F Duttenhoefer)

Background: Bone Morphogenetic Protein (BMP) binding endothelial regulator (BMPER) is a BMP modulator, similar to Chordin, Noggin or Gremlin and interacts with BMP 2, 4, 6, 7, 9 and 10. In humans, the syndrome Diaphanospondylodysostosis (DSD) is caused by a lack / mutation of the BMPER protein. Characteristics are absent or severely delayed ossification of vertebral bodies and other bone defects, a short broad thorax, a short neck and respiratory insufficiency. The severe bone phenotype suggests that BMPER plays a major role in osteogenesis. No studies have been performed to assess whether BMPER might be as osteoinductive as BMPs themselves nor whether BMPER is able to potentiate the osteoinductive effects of BMPs.
Goal: The aim of the study is to identify the osteogenic and chondrogenic potential effects of BMPER and to establish whether BMPER is promising as a new factor for promoting bone and/ or cartilage regeneration. As a first step the effects of BMPER on MSCs (Mesenchymal stem cells) will be investigated *in vitro*.
Results: BMPER transcript and protein levels were upregulated during osteogenic differentiation of human MSCs, whilst being downregulated in chondrogenesis. Treatment of cells undergoing osteogenic differentiation with recombinant human BMPER alone revealed some effect in promoting osteogenic differentiation, as shown by Alizarin Red staining and by the determination of *RUNX2*/*SOX9* ratio, two transcription factors critical in osteogenic differentiation. However, there is a high donor variation which is investigated to predict response to BMPER. BMPER treatment seems to have a slight influence on gene expression levels of *BMP2* and some BMP receptors, as *ACVR1*, *BMPR1A*, *ACVR2A*, and *ACVR2B*, while it shows no feedback effect on its gene expression and protein levels. The combination of treatment with BMPER and BMP-2 or BMP-6 were investigated. Recombinant BMPER combined with BMP-2 inhibited each other's positive effects on the *RUNX2*/*SOX9* ratio. For *RUNX2*, as well as the ratio to *SOX9*, BMP-6 did not prove to be beneficial. However, BMP-6 treatment leads to lower *PPARG* expression, which is a transcription factor associated with adipogenesis. The figure below shows Alizarin Red staining of monolayer after 21 days of osteogenic differentiation with recombinant human BMPER.

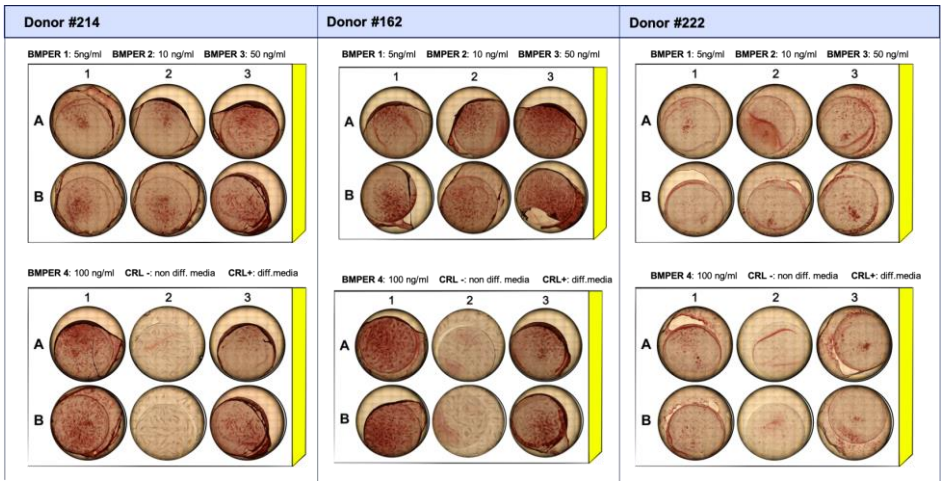


Figure 10.1.2 monolayer expanded human bone marrow MSCs from three different donors were cultures in the presence or absence of BMPER protein. Osteogenesis was assessed after 21 days by staining calcium deposition using alizarin red, with darker staining signifying more calcium

Biofunctional membrane for treatment of mandibular defect (Masticate) (Ongoing) (T Serra, D Eglin)

Background: In clinical practice the development of guided tissue regeneration has considerably influenced the possibility of implant use in the jaw regions with bone defects and those with a bone anatomy that is unfavorable for implant anchorage. Membranes used for guided tissue regeneration are used in combination with bone grafts or bone graft substitutes to support vertical bone augmentation, growth and closure of periodontal soft tissue. Still, dehiscence- and fenestration-type defects persist in a significant percent of patients. This may be due to difficulty in achieving primary wound closure and suboptimal speed of soft and hard tissue healing as a result of the large volume to be revascularized, covered and repaired.
Goal: The overall aim of this study is to develop a dual layer membrane using a surface acoustic wave additive manufacturing technology with 1) a bone layer made of assembled osteoconductive CaP microparticles into parallel lines in a collagen matrix and 2) a soft tissue layer made of pre-assembled adipose tissue-derived microvascular fragments within a collagen matrix, for fast vascularization and bone healing.
Results: A method for generating membranes based on CaP particles in collagen-based hydrogels was established (Figure 10.1.3a). A method for generating tissue engineered membranes with spatially organized microvasculature was established (Figure 10.1.3b) and published.

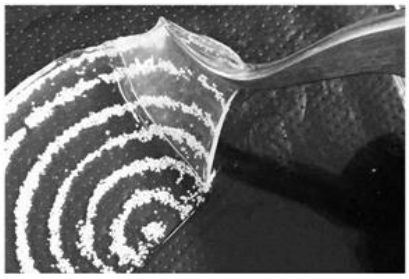
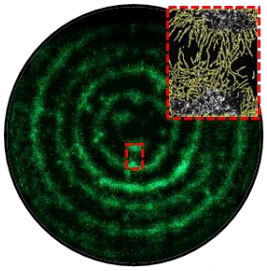
a  b 

Figure 10.1.3a) Example of gelatin-based hydrogel membrane loaded with a CaP particle pattern. 10.1.3b) Patterned vasculature. GFP-endothelial cells in green.

Pres:
11-15 December 2020. Acoustic waves cell patterning for spatially orchestrated vascular systems in tissue engineering. N Di Marzio, D Eglin, M Alini, T Serra. WBC 2020 World Biomaterials Congress. Virtual.

Pub:
Petta D, Basoli V, Pellicciotta D, Tognato R, Barcik J, Arrigoni C, Della Bella E, Armiento AR, Candrian C, Richards RG, Alini M, Moretti M, Eglin D, Serra T. Sound-induced morphogenesis of multicellular systems for rapid orchestration of vascular networks. (2020) Biofabrication 13 015004.

Inducing bone regeneration through immuno-modulation of biomaterials (RAIMBO) (Ongoing) (M D'Este, D Eglin)

Background: Bone repair in the craniomaxillofacial region is still a clinical challenge with a major impact on patients. For large defects after trauma or tumor resection, bone autografts are generally adopted as the clinical standard. However, this solution is far from ideal, as donor site morbidity and the limited amount of material available impose limitations. Bone graft substitutes from natural or synthetic biomaterials would be a valid alternative, although their efficacy is limited since they do not have the intrinsic healing properties of viable autologous bone. Additionally, they may still trigger deleterious immune responses, such as fibrous encapsulation, resulting in impaired new bone formation and possibly even leading to implant rejection. Adaptive and innate immune cells such as T cells, macrophages and neutrophils

play a central role in modulating the immune responses to biomaterials used in the fabrication of implantable devices. While some general principles governing the immune response to implanted biomaterials topography and chemistry have been investigated, the consequences of interactions of 3D printed constructs with the host immune system remains mostly unknown. The systematic knowledge of how variations in shape, topochemistry and composition of 3D printed constructs interacts with the host immune system opens the possibility of modulating the immune response to biomaterials, and ultimately to improve bone healing.

Goal: The overall goal of this project is to investigate the effect of specific material properties on immune cells such as neutrophils or macrophages. This will contribute to the design of biomaterials inducing a "healthy" inflammation in target clinical problems such as osteointegration; infection; fibrous encapsulation; osteolysis; implant rejection; new bone formation.

Results: a range of methods to characterize neutrophils response upon interaction with soft and hard biomaterials was established. The figure below illustrates a live/dead assay carried out depositing primary human neutrophils on bare tissue culture plastic (Plastic) compared with the same well plates coated with the tyramine derivative of HA (THA), composite THA-collagen (col), pure collagen, gelatin methacryloyl (GelMa), polyvinyl alcohol (PVA), poly caprolactone (PCL).

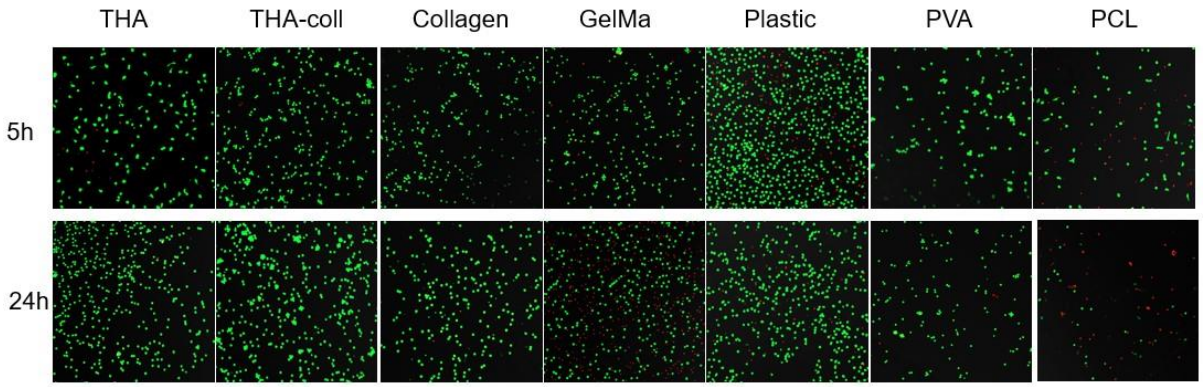


Figure10.1.4: Live and dead assay, illustrating living (green) and dead (red) primary neutrophils interaction with a range of biomaterials. THA: tyramine derivative of hyaluronan; coll: type I collagen; GelMa: Gelatin methacryloyl; Plastic: tissue culture plastic; PVA: poly vinyl alcohol; PCL: poly caprolactone.

Further experiments were carried out to determine myeloperoxidase activity, production of reactive oxygen species, metabolic activity, elastase. The results indicate that soft hydrogels from naturally derived polymers, especially HA, limit neutrophil activation. The consequences for the bone healing process are being assessed.

Pres:

Marinus A. Wesdorp, Andrea Schwab, Roberto Narcisi, David Eglin, Gerjo J.V.M. van Osch, Matteo D'Este; Assessment of the Neutrophil Response to a Panel of Synthetic and Natural derived Biomaterials; a Novel Comprehensive *in vitro* Approach. Oral presentation at the Netherlands Society for Biomaterials and Tissue Engineering, November 26 and 27 2020, held online.

Partners:

- Wesdorp T, The University Medical Center Rotterdam, Netherlands
- Narcisi R (PhD), The University Medical Center, Rotterdam, Netherlands
- van Osch G (Prof), The University Medical Center, Rotterdam, Netherlands

10.2 AOSpine

A translational approach integrating developmental biology and tissue engineering towards regeneration of the annulus fibrosus of the intervertebral disc (PrintDisc) (Started) (A Vernengo, Z Li, S Grad, M Alini)

Background: Intervertebral disc (IVD) degeneration can lead to severe and chronic low back pain, which represents the leading cause of disability worldwide. IVD degeneration can be characterized by the dehydration of the central nucleus pulposus (NP) and subsequent structural breakdown of the peripheral annulus fibrosus (AF). Currently, there are no clinically acceptable solutions for sealing the ruptured AF, so biologic based therapies, such as tissue engineering, are being investigated. The current state of AF tissue engineering may be advanced with a strategy that captures the essential building blocks of AF tissue morphogenesis: Biomimetic cell patterning, mechanical forces to induce circumferential cell elongation, and an extracellular matrix (ECM)-based molecular cocktail that supports tissue assembly.
Goal: Herein we aim for three important milestones: 1) The development of a novel, gentle method for preparation of AF decellularized ECM from bovine donors which preserves bioactivity. 2) The development of a free-form reversible embedding technique that supports biomimetic cell patterning, mechanical loading, and long-term culture of patterned cells. 3) The combination of these approaches in a 3D bioprinted construct that supports the spontaneous assembly of AF tissue with biomimetic histoarchitecture. The outcome of this work will be a platform which can be adapted to tissue morphogenesis of the central NP of the IVD, paving the way towards the goal of regenerating a whole tissue.

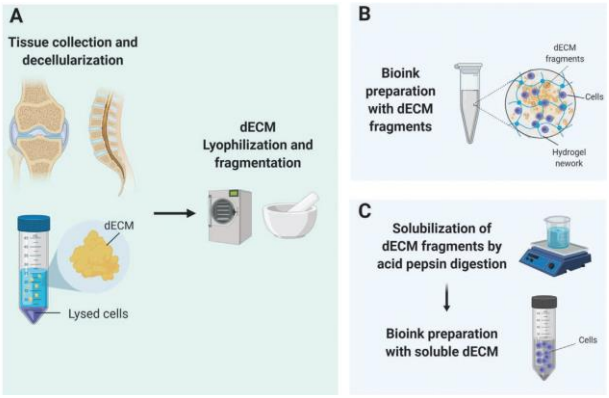


Figure 10.2.1: Overview of the decellularization and bioink preparation process. A) Tissue is decellularized by application of a detergent, enzyme, and/or physical disruption to induce cell lysis; dECM is lyophilized and fragmented via grinding or homogenization. Then, bioink is prepared by one of the two routes: B) Post-extrusion, shape retention is achieved with cross-linking of a biomaterial around the dECM fragments, forming a hydrogel. C) dECM fragments are solubilized via digestion by acid pepsin. The dECM pregel is prepared by combining cells with the solubilized dECM at cold temperatures and neutral pH. Postextrusion, the bioprinted dECM is heated to 37 °C to induce gelation via collagen assembly. From: Vernengo et al. doi: 10.1002/adfm.201909044.

Pub:
Alexeev D, Cui S, Grad S, Li Z, Ferguson S. Mechanical and biological characterisation of a composite annulus fibrosus repair strategy in an endplate delamination model. JOR Spine. 2020 Jul 16;3(4):e1107. doi: 10.1002/jsp2.1107. eCollection 2020 Dec.
Pfannkuche JJ, Guo W, Cui S, Ma J, Lang G, Peroglio M, Richards RG, Alini M, Grad S, Li Z. Intervertebral disc organ culture for the investigation of disc pathology and regeneration - benefits, limitations, and future directions of bioreactors. Connect Tissue Res. 61(3-4):304-321, 2020. doi: 10.1080/03008207.2019.1665652.
Vernengo AJ, Grad S, Eglin D, Alin M, Li Z. Bioprinting Tissue Analogues with Decellularized Extracellular Matrix Bioink for Regeneration and Tissue Models of Cartilage and Intervertebral Discs. Adv. Funct. Mater. 2020, 1909044. DOI: 10.1002/adfm.201909044.

Dissertation:
Kluser N. 3D Printed Multi-Scale Scaffolds with Topographical Guidance for Annulus Fibrosus Regeneration. 2020 ETH Zurich (A Vernengo, S Grad, S Ferguson) – MSc ETH HAST.

Evaluation of biological therapies and diagnostic targets for the degenerative intervertebral disc (Theranostic) (Started) (S Grad, Z Li, M Alini)

Background: Intervertebral disc (IVD) degeneration is a major factor contributing to the development of back and neck pain, which represents a global burden for patients, clinicians, and the society. For patients with early signs and symptoms who are not responsive to conservative treatment but do not yet qualify for spine surgery, therapeutic options are scarce. Recently, intradiscal injection of mesenchymal stromal cells (MSCs) derived from bone marrow has shown promising outcome in patients suffering from discogenic low back pain. The therapeutic effect of locally delivered MSCs has been attributed to their secretion of immune-modulatory, anti-inflammatory, and regenerative mediators. Direct application of MSC secretome is therefore an attractive therapeutic approach, avoiding some of the hurdles and risks of cell injection.
Goal: One aim of this project is to evaluate the potential of MSC secretome for IVD regeneration using cell and organ culture models. First, the secretome of human MSCs stimulated with different human IVD conditioned media will be characterized to assess the MSCs response to the IVD environment. Secondly, the therapeutic effect of stimulated MSCs' secretome on IVD cells and tissues will be investigated. Composition of the secretome will be correlated to its regenerative effect to identify potent secretomes for further pre-clinical study.

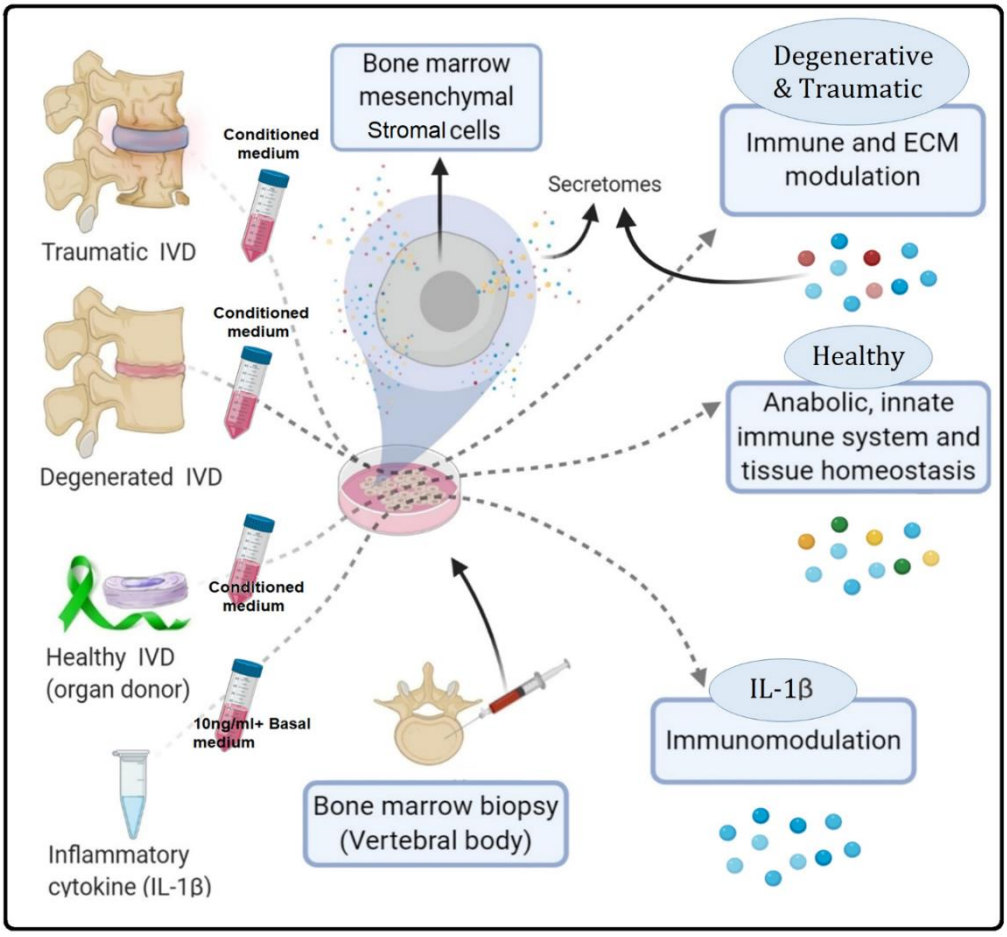


Figure 10.2.2: Scheme for generation of MSC secretome after stimulation with different conditioned media, and secreted MSC proteins in response to the different stimuli. From: Wangler et al. doi: 10.1186/s13287-020-02062-2.

Pres:
Russo F, Ambrosio L, Peroglio M, Wangler S, Guo W, Grad S, Alini M, Vadalà G, Papalia R, Denaro V. Human mesenchymal stem cells encapsulated in hyaluronic acid and platelet-rich plasma modulate matrix synthesis *ex vivo*. 2020 EORS virtual (poster).
Cui S, Zhou Z, Alini M, Grad S, Li Z. High impact loading organ culture model to investigate the post-traumatic disc degenerative condition. 2020 GR forscht virtual (oral).

Pub:
Du J, Pfannkuche JJ, Lang G, Creemers LB, Alini M, Grad S, Li Z. Proinflammatory intervertebral disc cell and organ culture models induced by tumor necrosis factor alpha. JOR Spine. 2020 Jun 19;3(3):e1104. doi: 10.1002/jsp2.1104. eCollection 2020 Sep.
Zhou Z, Cui S, Du J, Richards RG, Alini M, Grad S, Li Z. One strike loading organ culture model to investigate the post-traumatic disc degenerative condition. J Orthop Transl 2020.
Cui S, Zhou Z, Liu X, Richards RG, Alini M, Peng S, Liu S, Zou X, Li Z, Grad S. Identification and characterization of serum microRNAs as biomarkers for human disc degeneration: An RNA sequencing analysis. Diagnostics (Basel). 2020 Dec 8;10(12):1063. doi: 10.3390/diagnostics10121063.

10.3 AOTrauma

Temporal sequence of callus stiffening and mechanical callus induction limit (ActiveFixII) (Ongoing) (J Barcik, M Windolf)

Background: Despite decades of research on mechanobiology of fracture repair, certain aspects in the field remain untouched. While it is widely accepted that mechanical stimulation is required to promote callus formation, the minimum strain value that initiates callus formation remains unknown. Moreover, the impact of the temporal variation of mechanical stimulus is only barely understood. Several preclinical studies have provided conflicting results when attempting to quantify the impact of the temporal distribution of mechanical stimulation.
Goal: To further investigate the role of long-term stimulation timing (early versus delayed stimulation) together with the callus induction strain level using an established tilting wedge model.
Results: The tilting wedge experiment enables precise control of the stimulatory environment at the fracture gap. This stimulation is applied by an external pneumatic actuator. The model generates a strain gradient along the edge of the defect, thus permitting the quantification of callus response to different strain levels. The fixator was modified to apply a strain gradient from 0% to 20%. A control unit was designed to autonomously regulate the motion of the active fixator and to acquire the experimental data. After a series of *in vitro* tests, the active fixator was applied in two pilot sheep, executing an immediate stimulation protocol in one and a delayed stimulation protocol in the other. Both sheep tolerated the surgical procedure well and the fixator systems deliver seamless data over the course of the study. Evaluation of the preliminary data is currently ongoing.

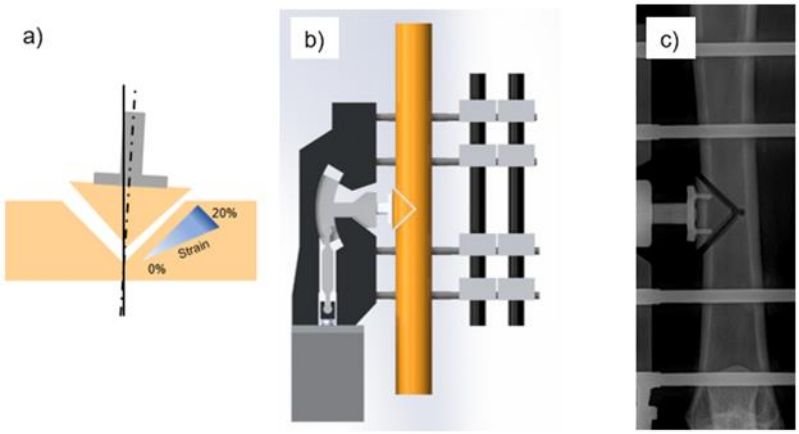


Figure 10.3.1: a) Operating principle of the tilting wedge fixator. The active fixator tilts the triangular bone fragment, thus creating a strain gradient from 0% to 20%; b) CAD model of the fixator; c) post-operative X-ray image of the implanted device in sheep tibia.

Pub:
Barcik J, Ernst M, Dlaska CE, Drenchev L, Zeiter S, Epari D R, Windolf M. Programable active fixator system for systematic *in vivo* investigation of bone healing processes. Sensors. 2021;21(1):17.
Barcik J, Ernst M, Schwyn R, Freitag L, Dlaska CE, Drenchev L, Todorov S, Skulev H, Epari DR, Zeiter S, Gueorguiev B. Development of surgical tools and procedures for experimental preclinical surgery using computer simulations and 3D printing. iJOE International Journal of Online and Biomedical Engineering. 2020;16:183.

Partner:
- Epari D (Prof), Queensland University of Technology (QUT), Brisbane, Australia

Predicting patient-specific mechanical failure of proximal humerus fracture plating with computer simulations (SystemFixII) (Ongoing) (P Varga, D Mischler, B Burkhard, D Ciric, VC Panagiotopoulou, C Schopper, M Remppis, M Windolf)

Background: The high failure rate of osteoporotic proximal humerus fracture fixations and the expected increase in their incidence indicate the need for improved fixation strategies and careful planning. Validated computer models have a high potential to complement or partially replace conventional biomechanical testing, expedite implant optimization and design, refine surgical guidelines, support decision making and allow patient-specific preoperative planning. Ultimately, simulations are expected to help improve patient outcomes in osteoporotic proximal humerus fracture treatment.
In the first project phase (SystemFixI), a virtual osteosynthesis test kit was developed to simulate proximal fracture plating and predict mechanical fixation failure. This tool was validated experimentally and utilized in a series of virtual pilot studies to indicate ways of improving the application of plates, to compare different implants and to optimize the implant design towards improved stability. However, the models have not yet been demonstrated to predict mechanical fixation failure in real clinical cases.
Goal: To extend the simulation tool application from virtual to the real clinical scenarios and validate it clinically by predicting the patient-specific risk of mechanical fixation failure.
Results: The clinical data collection is running at both study centers of Leuven and Innsbruck. Thirteen out of the planned 40 patients with PHILOS-plated proximal humerus fractures have been recruited. The methodology of creating subject-specific computer simulations from the clinical CT data has been developed and is currently being applied to predict subject-specific fixation failure risk of the patients. Moreover, a custom approach has been developed to evaluate postoperative shoulder activity of the subjects based on sensor data collected in a period of six weeks, with the aim to investigate whether the activity level and recorded shoulder angles could be related to the risk of fixation failure. Several sub-studies have been published during this project phase. One paper analyzed by utilization of the virtual osteosynthesis test kit in an *in silico* trial the optimal pattern of cement augmentation for locked plating of proximal humerus fractures. Two further prepares investigated screw perforation failure biomechanically and with detailed micro finite element models.

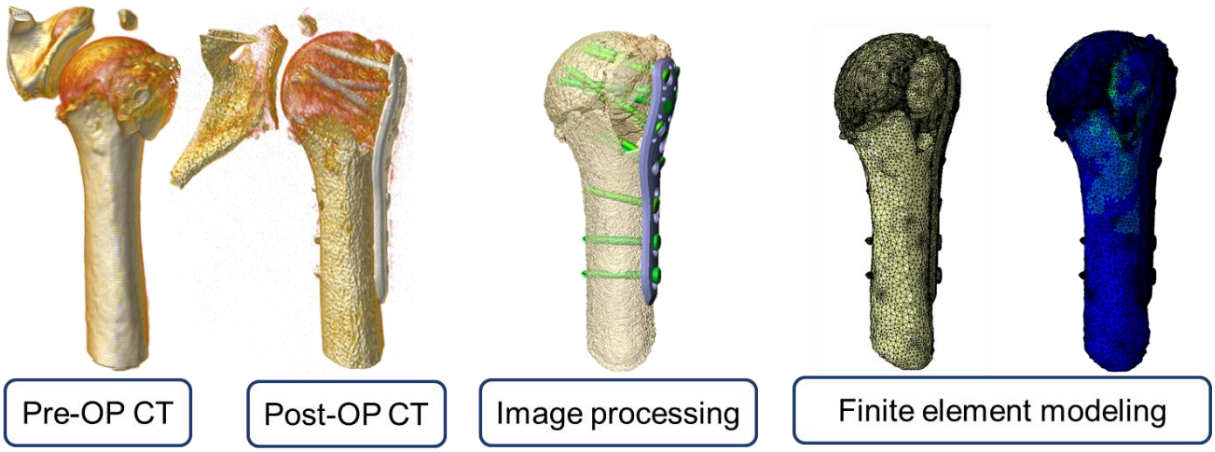


Figure 10.3.2: Subject-specific finite element simulation workflow

Pres:
Burkhard B, Schopper C, Ciric D, Mischler D, Gueorguiev B, Varga P. Cyclic perforation risk is increased by overdrilling in locked plating of complex proximal humerus fractures. 2020 EORS (oral).
Panagiotopoulou VC, Ovesy M, Gueorguiev B, Richards RG, Zysset P, Varga P. Micro finite element simulations accurately predict perforation of single screws in the proximal humerus. 2020 EORS (oral).

Varga P. *In silico* optimization of fracture fixations exemplified on the proximal humerus. Symposium: *In silico* musculoskeletal system modelling: potentials and perspectives. 2020 ESBioMech (invited oral).

Pub:
Mischler D, Babu S, Osterhoff G, Pari C, Fletcher J, Windolf M, Gueorguiev B, Varga P. Comparison of optimal screw configurations in two locking plate systems for proximal humerus fixation - a finite element analysis study. Clin Biomech. 2020;78:105097.
Varga P, Inzana JA, Fletcher JWA, Hofmann-Fliri L, Runer A, Südkamp NP, Windolf M. Cement augmentation of calcar screws may provide the greatest reduction in predicted screw cut-out risk for proximal humerus plating based on validated parametric computational modelling. Bone Joint Res. 2020;9(9):534.
Mischler D, Windolf M, Gueorguiev M, Nijs S, Varga P. Computational optimisation of screw orientations for improved locking plate fixation of proximal humerus fractures. J Orthop Translat. 2020;25:96.
Panagiotopoulou VC, Ovesy M, Gueorguiev B, Richards RG, Zysset P, Varga P. Experimental and numerical investigation of secondary screw perforation in the human proximal humerus. J Mech Behav Biomed Mater. 2021;epub.
Burkhard B, Schopper C, Ciric D, Mischler D, Gueorguiev B, Varga P. Overdrilling increases the risk of screw perforation in locked plating of complex proximal humeral fractures – a biomechanical cadaveric study. J Biomech. 2021;epub.

Partners:
- Nijs S (Prof), University Hospital Leuven, Belgium
- Hengg C (MD), Medical University Innsbruck, Austria

Evaluation of the biomechanical benefit of optimized patient-specific fixations for complex proximal humerus fractures (PSPH) (Ongoing) (D Mischler, JF Schader, J Dauwe, B Gueorguiev, P Varga)

Background: Treatment of complex proximal humerus fractures is still challenging with high complication rates, suggesting that the use of generic implants might not be sufficient to treat the high variability of patients and fractures. In a previous study, using a biomechanically validated computational framework, we demonstrated that optimizing the screw orientation of a common locking plate could significantly lower the predicted risk of cut-out failure.
Goal: To evaluate whether locking plates featuring optimized screw trajectories can provide significantly better biomechanical fixation stability compared to conventional implant design.
Results: Nine human cadaveric humeral pairs of elderly women were carefully selected from a larger sample set to minimize potential confounding factors such as bone mineral density variations and head size. Using 3D-printed specimen-specific cutting guides, the bones were symmetrically osteotomized to replicate a complex three-part fracture. The bones of each pair were randomly slit for plating with either conventional screw configuration or optimized screw orientation. All humeri were instrumented with 3D-printed metal plates featuring custom screw holes orientations according to the respective plate fixation. The specimens were cyclically tested with a gradually increasing peak force until cut-out failure. The constructs with optimized screw orientation sustained significantly higher cycles to failure, indicating a superior performance of the optimized screw trajectories compared to the standard implant design.

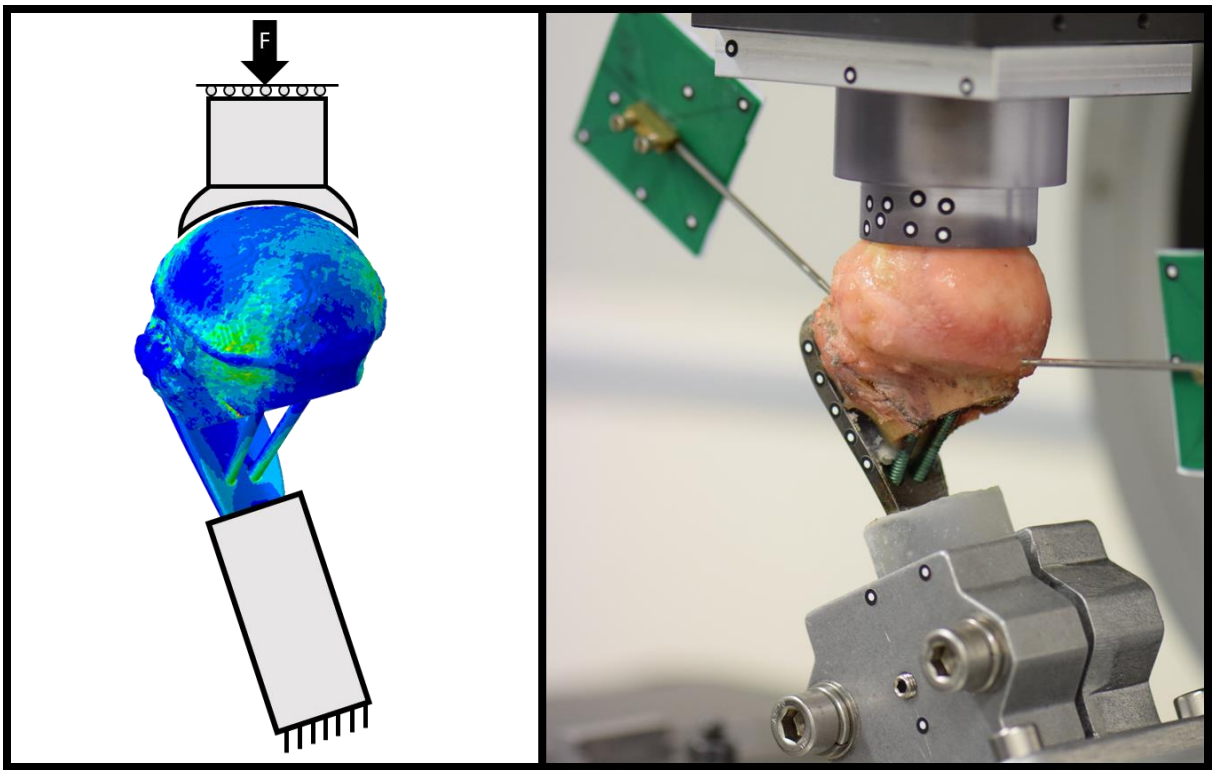


Figure 10.3.3: Finite element model (left) used to optimize screw orientations, together with test setup (right) used for its validation a paired human cadaveric study.

Pres:
Schader JF, Mischler D, Dauwe J, Gueorguiev B, Varga P. Patient-specific computational optimisation of the locking screw orientation of a proximal humerus plate in osteoporotic fracture fixation. 2020 EFORT (oral).
Schader JF, Mischler D, Dauwe J, Mys K, Gueorguiev B, Varga P. Improving stability of proximal humerus fracture locked plating by means of patient-specific computational optimization. 2020 AGA Kongress (poster).

Biomechanical evaluation of a new concept for screw-in-screw fixation of sacrum fragility fractures (SacrumFix) (I Zderic, B Gueorguiev)

Background: Surgical treatment of fragility sacrum fractures with percutaneous sacroiliac (SI) screw fixation is associated with high failure rates in terms of screw loosening, cut-through and turn-out. The latter is a common cause for complications, being detected in up to 20% of the patients.
Goal: To develop a new screw-in-screw concept and prototype implant for fragility sacrum fracture fixation and test it biomechanically versus transsacral and SI screw fixations.
Results: Twenty-seven artificial pelves with discontinued symphysis and a vertical osteotomy in zone 1 after Denis were assigned to three groups for implantation of their right sites with either an SI screw, the new screw-in-screw implant, or a transsacral screw. All specimens were biomechanically tested to failure in upright position with the right ilium constrained. Validated setup and test protocol were used for complex axial and torsional loading, applied through the S1 vertebral body. Interfragmentary movements were captured by motion tracking. Screw motions in the bone were evaluated by means of triggered anteroposterior X-rays. Interfragmentary movements and implant motions in terms of pull-out, cut-through, tilt, and turn-out were significantly higher for SI screw fixation compared to both transsacral screw and screw-in-screw fixations. In addition, transsacral screw and screw-in-screw fixations revealed similar construct stability. Moreover, screw-in-screw fixation successfully prevented turn-out of the implant that remained at 0° rotation around the nominal screw axis unexceptionally during testing. From a biomechanical perspective, fragility sacrum fracture fixation with the new

screw-in-screw implant prototype provides higher stability compared with the use of one SI screw, being able to successfully prevent turn-out. Moreover, it combines the higher stability of transsacral screw fixation with the less risky operational procedure of SI screw fixation and can be considered as their alternative treatment option.

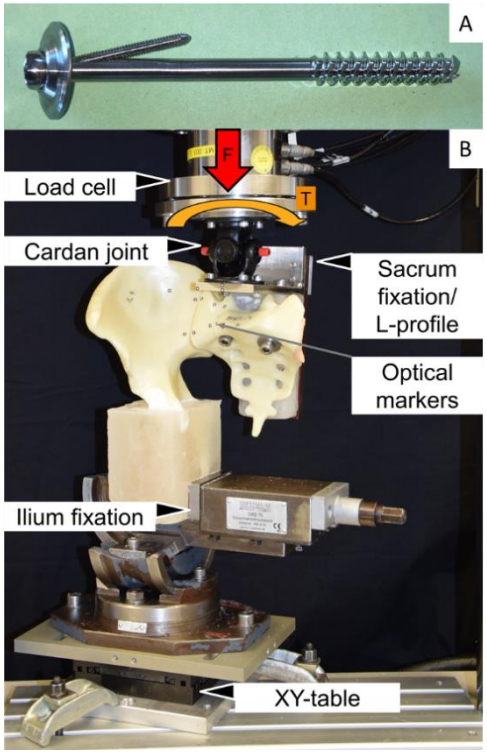


Figure 10.3.4: A) The screw-in-screw prototype, composed of a 7.3 mm cannulated screw, a 2.7 mm antirotation screw, and a washer. The angle between the two screws is 15°. The washer allows an angulation of up to 40°; B) Setup with a specimen mounted for mechanical testing. Vertical and semi-circular arrows show loading direction.

Pres:
Zderic I, Schopper C, Wagner D, Gueorguiev B, Rommens P, Acklin Y. Screw-in-screw fixation of fragility sacrum fractures provides high stability without loosening: a biomechanical study. 2020 EORS (oral).
Zderic I, Schopper C, Lodde M, Wagner D, Richards G, Gueorguiev B, Rommens P, Acklin Y. A new concept for screw-in-screw fixation of fragility sacrum fractures – biomechanical comparison versus transsacral and SI screw fixations. 2020 SGOT (oral).

Pub:
Zderic I, Wagner D, Schopper C, Lodde M, Richards G, Gueorguiev B, Rommens P, Acklin Y. Screw-in-screw fixation of fragility sacrum fractures provides high stability without loosening – a biomechanical evaluation of a new concept. J Orthop Res. 2020;epub.

Partners:
- Acklin Y (MD), University Hospital Basel, Basel, Switzerland
- Rommens P (Prof), University Medical Center Mainz, Mainz, Germany

Trabecular bone and cartilage analysis on osteoarthritic knees contrast enhanced CT images (CarCT) (Ongoing) (K Mys, D Gehweiler, P Antonacci, B Gueorguiev)

Background: Intra-articular fractures and osteochondral lesions frequently follow trauma accidents and are prone to progress into early osteoarthritis (OA). They are difficult to treat due to the poor healing capacity of the articular cartilage and have a high socioeconomic effect. OA affects nearly 10% of the whole population and 50% of the elderly. The treatment of osteochondral articular defects is important to prevent development of osteoarthritis. Therefore, improved visualization of the whole joint regeneration process is an important factor for targeting better treatment outcomes and development of new therapeutic procedures.
Goal: To quantify cartilage and underlying bone on high-resolution CT scans of fresh-frozen human cadaveric knees.
Results: Six human cadaveric knees were scanned twice by means of normal CT and contrast enhanced CT using ClinicalCT, XtremeCT and MicroCT scanners. Following, the condyles were automatically segmented and cortical, trabecular and cartilage layers were automatically detected with an in-house developed Matlab script. In the next project phase the layers will be processed and segmented with in-house developed advanced segmentation techniques to calculate cortical, trabecular and cartilage parameters and compare these results from *in vivo* scanners (Clinical CT and XtremeCT) versus the gold standard MicroCT.

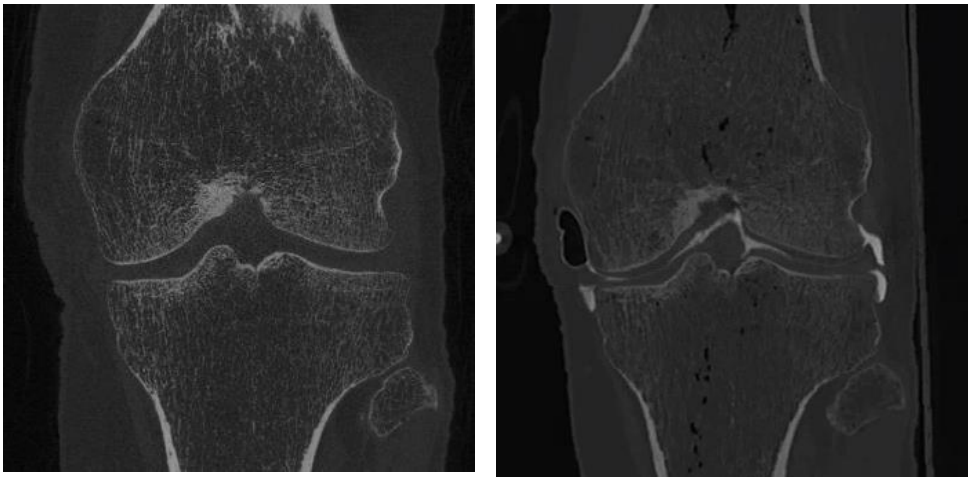


Figure 10.3.5: XtremeCT scan of a knee (left) and contrast enhanced clinical CT scan of the same knee (right).

Morphology of periclavicular space following dislocated fracture healing (PeriClav) (Ongoing) (D Gehweiler, E Zweifel, B Gueorguiev)

Background: Clavicle fractures are common sport-related injuries, especially among younger patients. The most common type is the displaced Edinburgh 2B midshaft fracture. Nonoperative treatment of midshaft clavicle fractures can result in malunion with consequent changes in length, shape, and orientation of the lateral fragment. Since the position of the scapula is determined by the clavicle, the resting posture and movement of the scapula is altered by clavicular malunion, which can lead to impairments for the patient. The borders of the periclavicular space are defined by the clavicle, the scapula, the medial parascapular suspensory muscles, and the first rib. The periclavicular space is a conduit for the brachial plexus and subclavian-axillary vascular system. Changes in the shape and form of the periclavicular space generated by alteration in the anatomy of its bounding structures cause distortion of the plexus and vascular structures, particularly during motion of the upper extremity, and may typically result in variable clinical presentations of the so-called thoracic outlet syndrome. It remains unknown what the magnitude, or combination of the linear, angular, and rotational displacements of the lateral fragment of a simple midshaft clavicle fracture must be to cause neurovascular symptoms.

Goal: To understand and define 1) the periclavicular space as a function of the arm and shoulder position and movement, and 2) the relationship between clavicular dysmorphology and the volume and shape of the periclavicular space with respect to the first rib by simulation of common combinations of clavicular malunion.
Results: A custom-made radiolucent frame was built that allows an upper torso to be fixed upright so that the shoulder can move freely in all planes of motion at the isocenter of a clinical CT scanner. A first series of scans with an intact clavicle was performed for one specimen. All scans were processed so that the first rib serves as a fixed reference in space. Clavicula, scapula and humerus were registered to match the position within the respective scans.

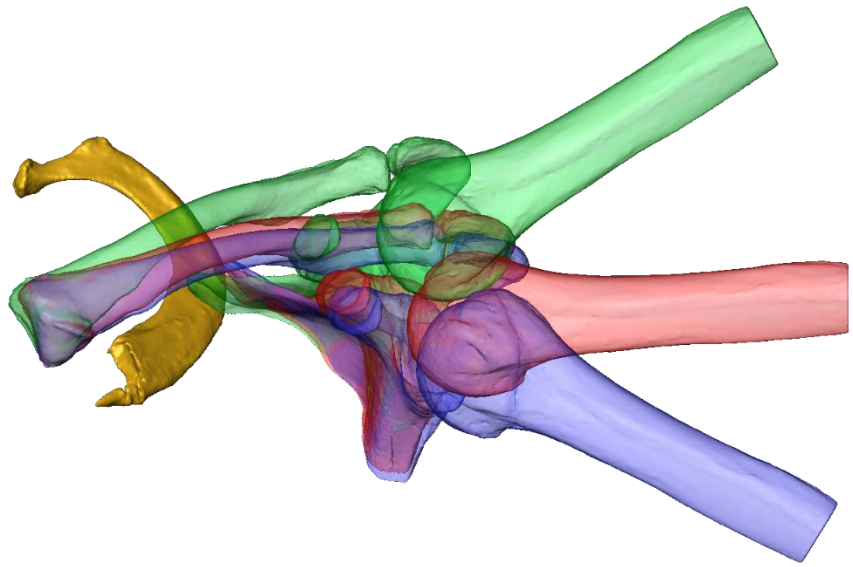


Figure 10.3.6: Illustration of a left shoulder during motion in the CT scanner: first rib (yellow) serves as reference; clavicle, scapula and humerus colored blue at 60° abduction, red at 90° abduction and green at 120° abduction.

Partners:
- Lambert S (MD), University College London Hospital, London, UK
- Jaeger M (MD), University Medical Center Freiburg, Freiburg, Germany

Optimization of a subcutaneous infection model in mice (Submurine) (Ongoing) (C Wittmann, F Moriarty, S Zeiter)

Background: Fracture related infection (FRI) is one of the most common and feared complication in fracture management. To evaluate any new interventional strategy, it is essential to utilize standardized and reproducible models to generate robust and comparable results. One of the used models is a subcutaneous implant infection mode in the mouse, suited to screening new technologies at an early stage of assessment. It currently lacks important aspects such as the impact of prophylactic antibiotics, as well as the inclusion of a positive control i.e. the current gold standard, to be able to better interpret the obtained results.
Goal: This project aims to optimize and better characterize this model.
Results: Ongoing.

miRNA analysis to discover fracture related biomarkers (MiDiag2) (Started) (M Stoddart, M Alini, H Schmal)

Background: Biomarkers predictive of fracture healing outcomes would provide a useful tool to allow surgeons to proactively make patient based clinical decisions. Currently, even in high-risk groups, there are no accurate way to determine the potential of a particular patient to progress to delayed or non-union. Such a tool would enable more reliable patient stratification, thus allowing for earlier diagnosis and increasing the potential success of additional early interventions by the surgeon. In a previous AOTrauma project (MiDiag) we investigated changes in small non-coding RNA in fracture patients and during osteogenic differentiation.
Goal: A panel of prospective microRNA (miRNA) markers were identified, and this now requires further validation. In addition, we aim to use the methods developed, and the database of patient serum non-coding RNA created, to further investigate markers that are mechanically regulated and would be associated with secondary bone healing.
Results: Mechanically induced chondrogenesis is exploited to analyze *in vitro* which are the miRNAs involved into callus formation, the first step to endochondral ossification and fracture healing. A cartilage bioreactor was used to investigate early mechanically induced chondrogenesis (3 days) and identify differentially expressed miRNA by RNA sequencing. Early markers of transforming growth factor – β (TGF-β) signaling activation or of differentiation were investigated. Long term experiments are also ongoing to correlate miRNA expression to differentiation outcomes.

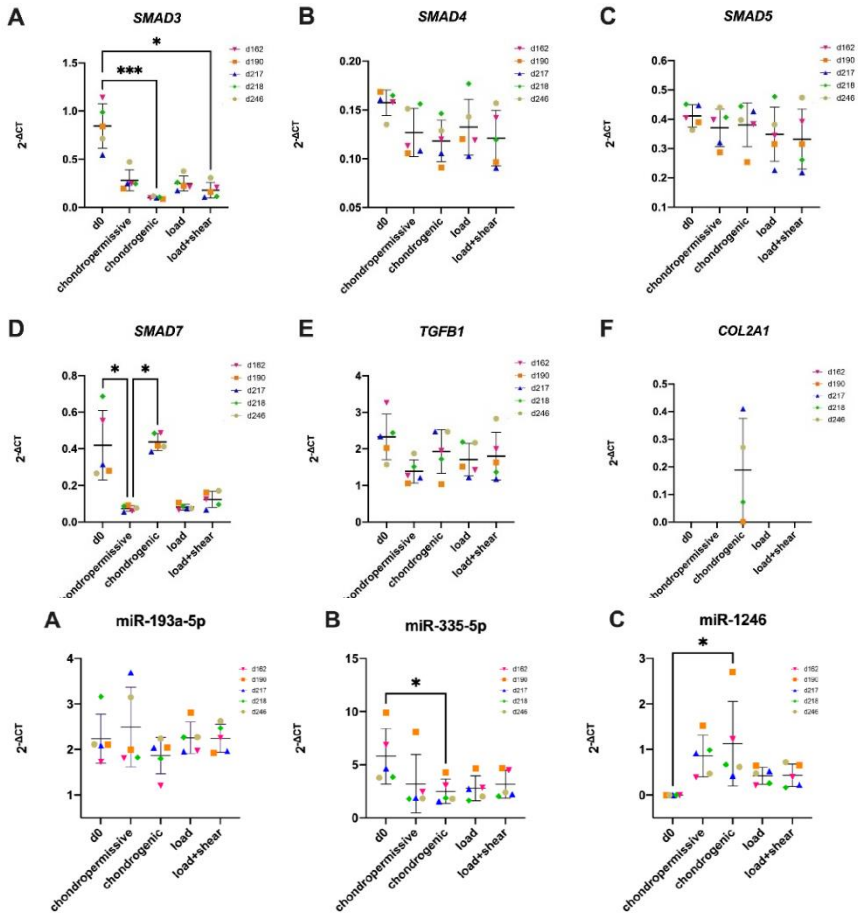


Figure 10.3.7: shows results about the expression of SMADs and of three selected miRNAs, as identified by the previous miDiag project.

Pub: P Ahmad, M Stoddart, E Della Bella. The role of noncoding RNAs in osteogenic differentiation of human periodontal ligament stem cells. *Craniomaxillofacial Trauma and Reconstruction Open* 2021. In press.

Partner:

- Kubosh EJ (Dr), Department of Orthopedics and Traumatology, University Medical Center Freiburg, Germany

Immunoprofiling of fracture patients to determine predictive biomarkers of healing (NUPredict) (Ongoing) (K Thompson, MJ Stoddart, M Alini)

Background: In the context of fracture repair, a significant number of patients (≤10%) continue to display healing deficiencies, despite marked advances in the understanding of fracture healing. Although specific co-morbidities such as diabetics and smokers may increase risk of delayed healing and potential progression to non-union, currently it is not possible to identify specific patients at elevated risk of delayed healing. A number of recent studies have indicated that specific populations of blood-resident immune cells are altered in patients with diminished fracture healing. These immune cell changes act to inappropriately maintain a pro-inflammatory environment that negatively affects bone regeneration. In addition, certain non-coding microRNA signatures have also been identified in the blood of non-union patients, suggesting that profiling of such markers may be a means to identify patients at risk of non-union and allow earlier therapeutic interventions when healing capacity may be recovered. However, the majority of these reported studies are hampered by small sample numbers, meaning that the applicability of such assays to identify non-union patients currently requires further investigation.
Goal: To conduct in-depth immunophenotyping of blood-resident immune cells in delayed healing patients compared to normal healing patients. In addition, we seek to identify whether specific profiles of blood-resident miRNAs can be used to identify delayed healing patients.
Results: To date we have identified a number of changes in both circulating levels of specific T cell subsets and miRNA signatures in 12 non-union patients compared to 8 normal healing patients. The functional consequences of such changes in immune cell proportions and their correlated surface marker expression, such as immune cell-mediated production of pro- and anti-inflammatory cytokines, are now being further investigated and assessed versus clinical parameters. In addition, a panel of miRNA biomarkers are now the subject of a further validation in a wider patient cohort to determine whether such changes correlate with healing outcome.

The influence of the gut microbiota on bone (BacBone2) (Started) (A Wallimann, K Thompson, S Zeiter, F Moriarty)

Background: It has become evident, that the gut microbiota plays a crucial role in many diseases, including bone-associated morbidities, and possibly also fracture healing. One mechanism for gut microbiota interaction with bone is through production of metabolites, especially short-chain fatty acids (SCFAs).
Goal: In this project, we are investigating the impact of the gut microbiota on bone and the impact of changes in the microbiome (through probiotic supplementation and antibiotic therapy) on fracture healing.
Results: Butyrate is one of the most studied SCFA and we have found that butyrate inhibits murine, as well as human osteoclast formation *in vitro*. The effects of butyrate on bone was further explored in a murine bone-osteotomy model when butyrate was given as as upplement. In addition, mice were exposed to antibiotic therapy to determine the impact of microbiote disruption on bone healing in the presence and absence of butyrate. Antibiotics induced clear changes in the gut microbiota of the mice (Figure 10.3.8) and reduced SCFA (acetate, propionate and butyrate) levels in the cecum compared to control and butyrate treated mice (not shown). These results indicate the significant potential for microbiota-related interventions to positively or negatively influence fracture healing.

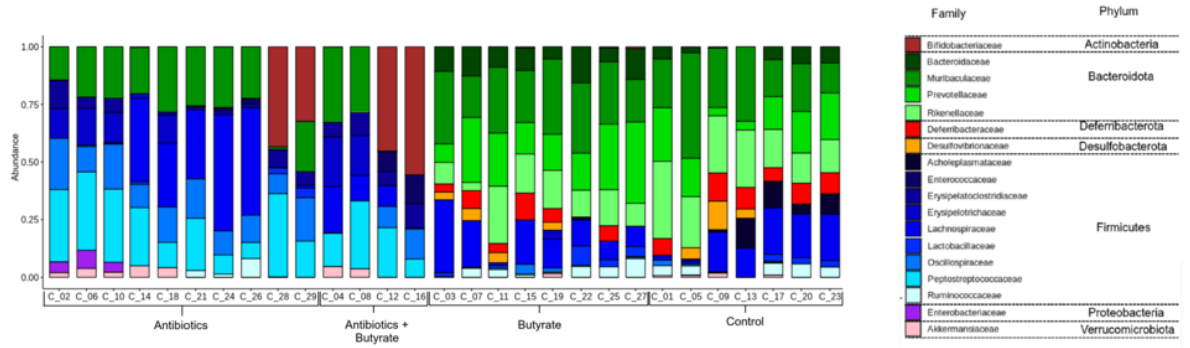


Figure 10.3.8: Gut microbiota of antibiotic treated mice (left hand side) clearly separates from control and butyrate treated mice (right hand side).

Pres:
"The influence of short-chain fatty acids on bone health" at Digital Graubünden Forscht Congress 23-24 September 2020 and at Digital WIRM Davos, 4-7 October 2020.
"The influence of microbial-derived metabolites on bone health" at EAACI Winter School 23-26 January 2020, Chamonix, France. Poster award for an outstanding poster presentation at EAACI Winter School 23-26 January 2020, Chamonix, France (poster).

Pub:
An exopolysaccharide produced by Bifidobacterium longum 35624® inhibits osteoclast formation via a TLR2-dependent mechanism. Calcified Tissue International, December 2020.

Partners:
- Akdis C (Prof), Swiss institute for Asthma and Allergy Research (SIAF) Davos, Switzerland
- O'Mahony L, University College Cork, Ireland

Implant retention in a sheep fracture related infection model: evaluating fracture healing and an antibiotic loaded hydrogel (DAIR) (Started) (C Siverino, F Moriarty, M Windolf, S Zeiter)

Background: Management of fracture related infection (FRI) often involves further surgical procedures with poor long-term outcomes. Debridement, Antibiotics, Irrigation and implant Retention (DAIR) is a surgical treatment protocol often applied to acute/early FRI. Clinically relevant pre-clinical models of DAIR in FRI are scarce and none have been developed in large animals.
Goal: Therefore, this project aims to develop a large animal model for FRI including a DAIR approach. Moreover, to increase the treatment success of DAIR in FRI, a new ARI-developed hydrogel containing antibiotics will be applied during revision surgery. Additionally, the fracture healing over time will be monitored using the ARI fracture monitor sensor.

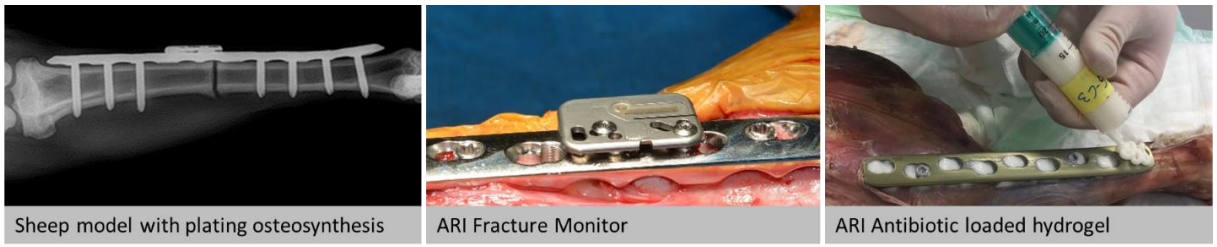


Figure 10.3.9: Overview of DAIR project, which aims to establish a large animal model for DAIR in acute S. aureus FRI, including monitoring of fracture healing and intervention with an antibiotic loaded hydrogel.

Results: The first outcomes prove that it is possible to establish a model of FRI with plating osteosynthesis. Analyses of the samples show high bacterial burden at revision and still traces of infection at euthanasia. Using the fracture monitor it will be possible to correlate the infection to the fracture healing.

Evaluation of an enzybiotic regimen for the treatment of fracture-related infection (Enzybiotic) (Ongoing) (F Moriarty, E Sumrall, S Zeiter)

Background: Fracture related infection (FRI) presents a significant challenge to the field of orthopedic trauma surgery. There is a need for novel antimicrobials that can readily target metabolically inactive, and antibiotic resistant *S. aureus* biofilms. Enzybiotics are a novel class of antimicrobial enzymes, naturally derived from bacteria and bacteriophages, which have been demonstrated *in vitro* and in animal models to be highly effective in preventing infection. As digestive enzymes, they are known to be particularly effective in the eradication of metabolically inactive (persister) cells, antibiotic resistant bacteria, and even biofilm matrix components.
Results: In this study, we have shown that an enzybiotic combination shows significant anti-biofilm effects in several *in vitro* models. Particularly, we showed in an *in vitro* infected implant model that an enzybiotic combination is particularly effective at eradicating *S. aureus* biofilms from three different strains (Figure 10.3.10). The enzybiotic combination was superior to an antibiotic treatment and showed significant additive effects when combined with antibiotics. This suggested that this enzybiotic combination could be effective at treating infection in an infected animal model of implant-associated infection. We therefore tested it in a mouse model and preliminary results show that locally-applied enzybiotics were increasing the efficacy of a vancomycin/gentamicin treatment**.**

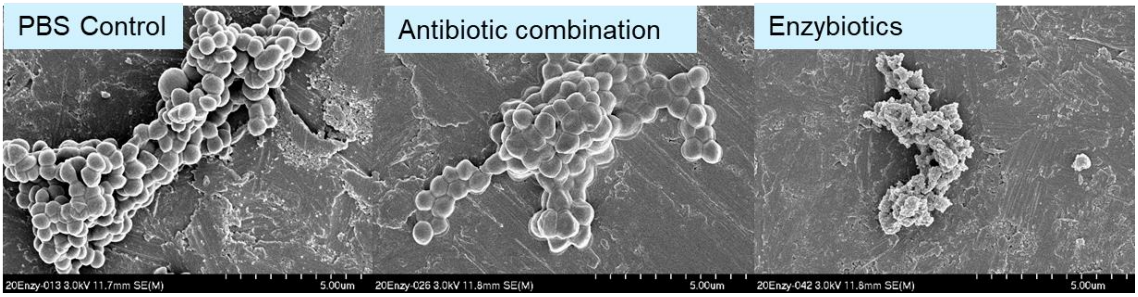


Figure 10.3.10: Scanning electron microscopy images of an in vitro implant biofilm exposed to saline (left), antibiotics (center) or enzybiotics (right). Note the normal appearance of the bacteria under control and antibiotic exposed conditions, but obvious destruction in the presence of enzybiotics.

Partner:

- Schmelcher M, Department of Health Sciences and Technology, ETH Zurich, Switzerland

Exopolysaccharide coated material surfaces modulating fracture immune status and enhancing bone healing (EPSIm) (Started) (F Moriarty, D Eglin)

Background: Bone healing complications such as delayed healing or non-union affect 5-10% of patients with a long bone fracture and lead to reduced quality of life and increased health care costs. The gut microbiota and its assortment of surface associated polymers and secreted metabolites have been shown to impact nearly all organs of the human body including bone.
Goal: In this study we look to investigate the effect of extracellular polysaccharide produced by Bifidobacterium longum subsp longum 35624 on cell types involved in fracture healing, such as osteoblasts, osteoclasts, chondrocytes, and fibroblasts, as well as indirectly, by shaping an appropriate anti-inflammatory and immune regulatory response.

Results: Methodologies for its production and purification have been established in the past year, with good yields now achieved. Methodology and experiments to verify its purity will be conducted in the near future. Other future steps will include coating the surface of orthopedic devices to determine its effect on host cellular responses *in vitro* and *in vivo*. The surface coatings properties and degradation profiles will also be characterized. A literature review investigating bacterial exopolysaccharides, their production and effect on bone biology and immunoregulation is in preparation.

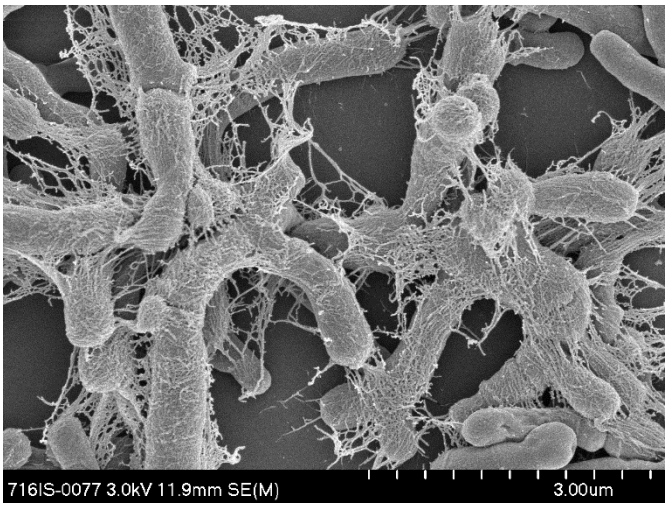


Figure 10.3.11: Scanning electron micrograph of Bifidobacterium longum with extracellular polymeric substances forming a meshwork around the bacteria.

Partners:

- Eglin D, Ecoles des Mines Saint Etienne, France
- O'Mahony L, University College Cork, Ireland

Local delivery system for improving efficacy of bacteriophages combined with antibiotics (Gelphage) (Ongoing) (M D'Este, F Moriarty)

Background: The use of medical devices such as fracture fixation devices has had an enormously positive impact on patient care. However, approximately 5% of patients, across all medical specialties, can develop an infection associated with the device, which can have disastrous consequences. These bacterial infections involve biofilm formation and are therefore always highly antibiotic tolerant, even in the absence of specific antibiotic resistance genes. The antibiotic recalcitrance of the biofilm leads to poor treatment success rates and often requires implant removal to treat the infection.
Goal: The aim of this project is to develop an injectable local delivering system for bacteriophages in combination with antibiotics and assess the efficacy of the combination of these potent bactericidal solutions in the treatment of antibiotic resistant biofilm caused by *Staphylococcus aureus*.
Results: Early investigations have determined the interaction of a commonly available bacteriophage (ISP) and the anti-staphylococcal antibiotic daptomycin. Using the checkerboard synergy test, we could see that this combination had a slightly synergistic effect whereby planktonic bacteria were more efficiently killed by this combination than expected based on individual activities. Future studies will look to test additional combinations including newly isolated phages against the most clinically relevant antibiotics to identify combinations with greater potency against biofilms. Furthermore, a number of biomaterials are being evaluated for their ability to deliver bacteriophage and antibiotics, maintaining both activity of both active agents, and retaining the synergistic interaction.

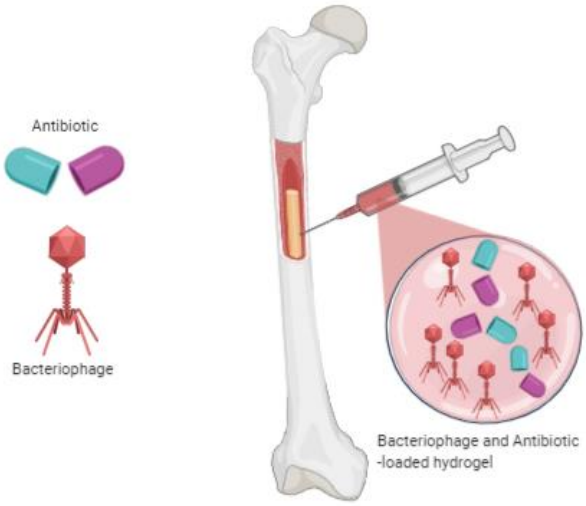


Figure 10.3.12: Overview schematic of the target for the Gelphage project: an injectable hydrogel loaded with antibiotics and bacteriophage designed to prevent and treat fracture-related infection.

Pub:

Rotman SG, Sumrall E, Ziadlou R, Grijpma DW, Richards RG, Eglin D, Moriarty TF; Local Bacteriophage Delivery for Treatment and Prevention of Bacterial Infections. Front Microbiol, 2020, 11, 2276.

Pérez-Köhler B, Pascual G, Benito-Martínez S, Bellón J M, Eglin D, Guillaume O; Thermo-responsive antimicrobial hydrogel for the in-situ coating of mesh materials for hernia repair. Polymers, 2020, 6, 1245.

Development of 3 dimensional *in vitro* models of bone infection (Immunobact2) (Ongoing) (F Moriarty, M Hofstee, S Zeiter)

Background*:* *Staphylococcus aureus* is a prominent human pathogen in bone infections. Within bone marrow, *S. aureus* pathophysiology involves abscess formation, which consist of central staphylococcal abscess communities (SACs), surrounded by a fibrin pseudocapsule and infiltrating immune cells.

Goal: One objective of the project involved the development of a 3D *in vitro* SAC model that could replicate the *in vivo* situation.

Results: In a murine study performed in ARI, SACs were observed within the bone marrow of infected mice with fracture-related infection (Figure 10.3.13, left hand side). The *in vivo* abscesses were encapsulated by fibrin (magenta stained structure), collagen and myofibroblasts, with regulatory T cells and M2 macrophages at the periphery (not shown). To this goal, SAACs have been formed in a human plasma supplemented collagen gel (Figure 10.3.13, right hand side). The 3D *in vitro* SACs closely resembled *in vivo* SACs and tolerated 100x the minimum inhibitory concentration of the antibiotics gentamicin and rifampicin. Furthermore, primary human neutrophils were unable to clear mature *in vitro* SACs.

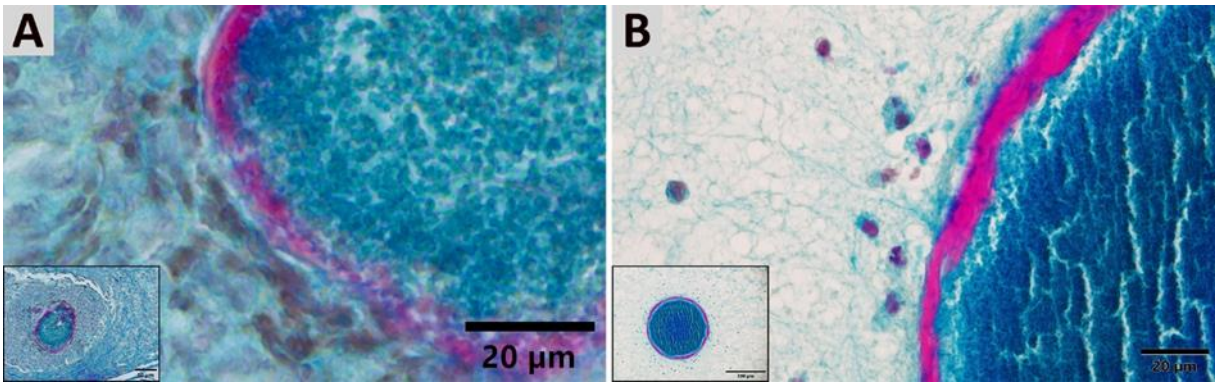


Figure 10.3.13: Morphological comparison between (A) in vivo staphylococcal abscess communities in mice (SACs) and (B) in vitro SACs stained with a Picro-Mallory trichrome stain where connective tissue and mucopolysaccharides stain blue, muscles and erythrocytes stain yellow, and fibrin stains magenta. Nuclei of cells are stained purple/brown. Scale bars: (A and B) 20 µm.

Pres:
M Hofstee, A 3-dimensional *in vitro* Staphylococcus aureus abscess community model. Graubünden Forscht 7th conference, 23&24 September 2020, virtual.

Pub:
MI Hofstee, G Muthukrishnan, GJ Atkins, M Riool, K Thompson, M Morgenstern, MJ Stoddart, RGRichards, SAJ Zaat, TF Moriarty, Current concepts of osteomyelitis: from pathological mechanisms to advanced research methods. *The American Journal of Pathology,* 190, 1151-1163 (2020).
MI Hofstee, M Riool, I Terjajevs, K Thompson, MJ Stoddart, RG Richards, SAJ Zaat, TF Moriarty, Three-dimensional *in vitro* Staphylococcus aureus abscess communities display antibiotic tolerance and protection from neutrophil clearance. *Infection and Immunity*, 88, e00293-20 (2020).

Partner:
- Zaat SAJ (Dr), Amsterdam UMC location AMC, Amsterdam, Netherlands

An *in vitro* model to study *S. aureus* invasion of the osteocyte lacuno-canalicular network (InViBo, Feasibility) (Ongoing) (C Siverino, F Moriarty)

Background: *S. aureus* induces chronic fracture related infection (FRI) by forming biofilm, by infecting bone cells and surviving intracellularly, but also by invading the osteocyte lacuna-canalicular network (OLCN). In the OLCN, the bacteria are able to deform their shape to propagate inside the narrow canaliculi. This mechanism allows the bacteria to escape from the immune system and persist inside the bone causing increasing the failure rate for the treatment of FRI.
Goal: Establishment of a new *in vitro* model to study bacterial invasion of the OLCN.
Results: Bone cores were isolated from sheep femur and were either decellularized or underwent freeze-thaw cycles or were cultured *ex-vivo*. Outside surfaces of the bone cores after isolation were analyzed by micro-computed tomography. Efficacy of decellularization and of the freeze-thaw cycles were confirmed by DNA quantification. Cell viability of the *ex vivo*-cultured bone cores was monitored over 14 days. Incubation of decellularized or freeze-thawed bone cores with *S. aureus* for 1, 3, and 7 days showed increasing infiltration of the bacteria into the bone depth.

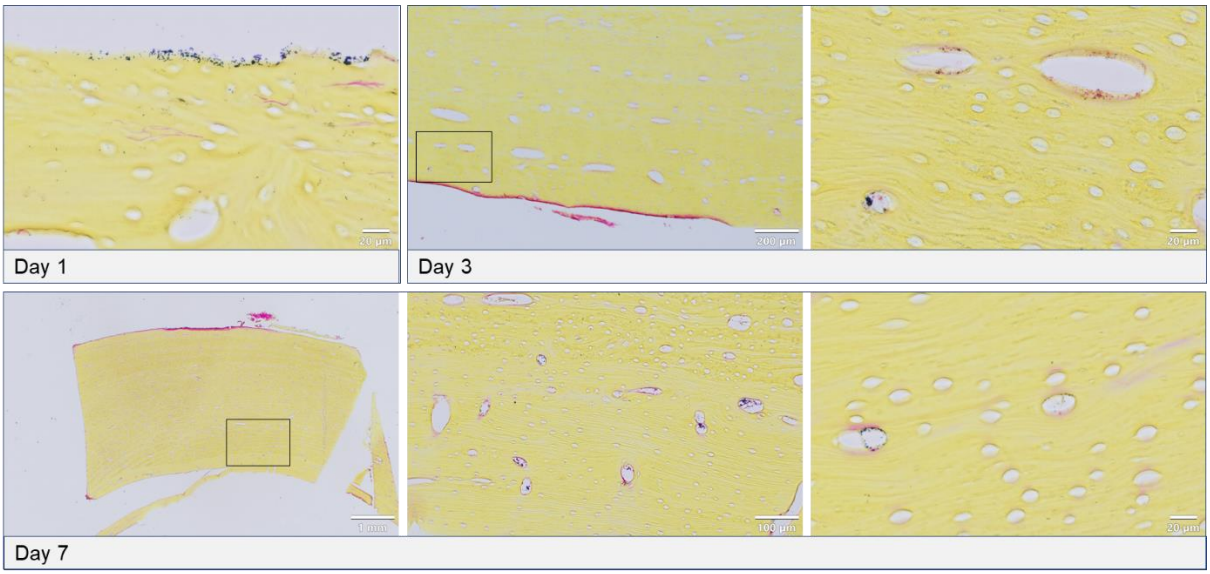


Figure 10.3.14: Brown and Brenn staining of decellularized bone cores incubated for 1, 3 or 7 days with S. aureus (blue) confirming infiltration of bacteria into the bone.

Ultimately, with this new *in vitro* model, we can investigate which antimicrobial treatments are more efficient against bacteria in this niche and determine if other pathogens also display this behavior.

Bone defect healing after chronically infected non-union (MASCOT) (Ongoing) (C Siverino, F Moriarty, S Zeiter)

Background: In chronically infected non-unions, treatment always includes extensive debridement to remove necrotic and infected bone, often resulting in large defects requiring elaborate and prolonged bone reconstruction techniques.
Goal: In this project, we aim to investigate if the inflammation induced by the infection may persist locally after the bacteria have been killed and if a prolonged infection leads to scarring, bone sclerosis, lysis, and poor blood supply that in combination limit bone regeneration in the defect.
Results: First, we established a rabbit humerus model (non-infected) with 5 mm plating osteosynthesis that includes the induced membrane technique (IMT) and bone grafting. The development of this preclinical *in vivo* model will be a valuable resource to explore treatment concepts of infected non-union and facilitate studies into the many clinically-driven questions behind current treatment concepts.

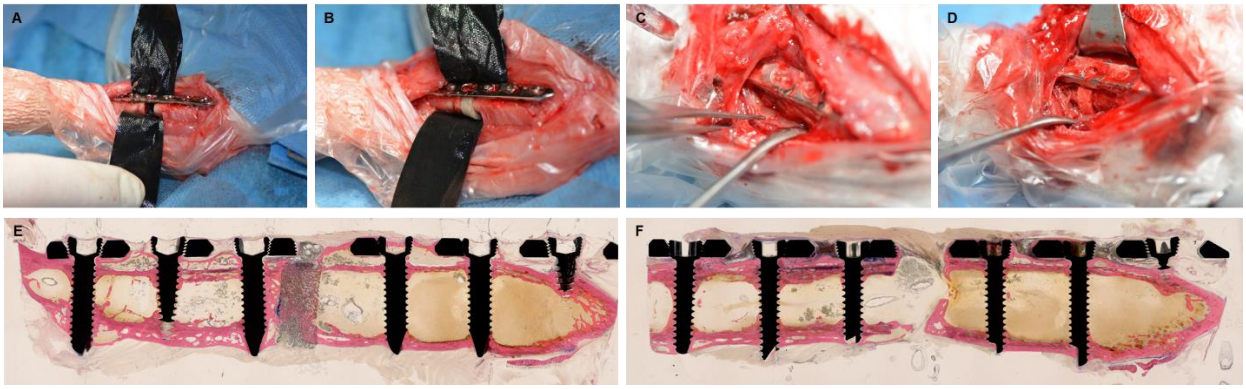


*Figure 10.3.15: Rabbit humerus model of IMT and histological evaluation - **A** 5 mm empty humerus defect after surgery stabilized with the 2.4 mm plate; **B** PMMA spacer filling the 5 mm osteotomy; **C** revision surgery after 3 weeks shows the presence of induced membrane over the PMMA spacer; **D** chronOs inserted at the osteotomy site; G&E staining of bone formation after 12 weeks from revision **E** with chronOs compared to **F** empty defect.*

Identification of microRNA biomarkers associated with Staphyloccocal fracture-related infection (Xtra-Bac, Feasibility) (Ongoing) (F Moriarty, M Stoddart, E Sumrall)

Background: MicroRNAs are a class of non-coding RNAs that play key roles in the regulation of gene expression. Of specific relevance for bone infections, microRNAs released by infected cells could play key roles in pathogen-host interactions and may serve as diagnostic biomarkers of disease progression.
Goal: our goal was to perform a non-biased, global microRNA analysis using RNA-seq, with a diagnostic perspective.
Results: Our data were generated from a population of 20 human patients with orthopedic device related infection, caused by either *S. aureus* (n=10) or *S. epidermidis* (n=10) and compared to 20 uninfected patients with orthopedic devices (controls). Analyses of patient samples with both types of infection showed a significant number of miRNA sequences enriched in the blood serum relative to uninfected patients (Figure 10.3.16). Top miRNA targets were selected based on a high absolute fold-change in expression in infected samples relative to uninfected samples, with a high level of consistency (low P-value). Of the large number of significant targets in both types of infection, 8 miRNA targets were common to both *S. aureus* and *S. epidermidis* infections. These targets, as well as a comparison of the fold-change levels in both infection types, are depicted in Figure 10.3.16. We hope that a further study will allow us to narrow down this list of targets to yield a clear panel of targets with a high diagnostic power.

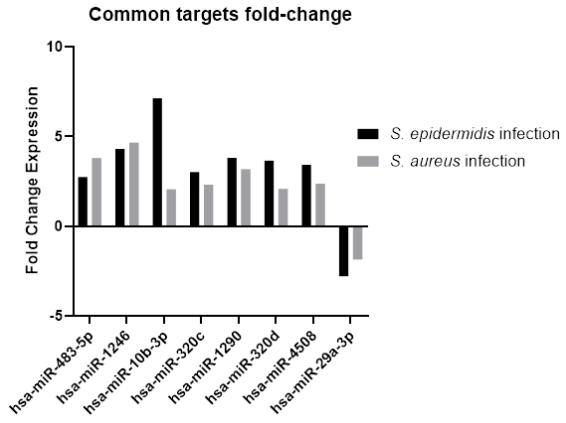


Figure 10.3.16: Fold-change expression of the 8 miRNA targets (X-axis labels) demonstrating significant differential expression levels in both S. aureus and S. epidermidis bone infection. A negative fold-change indicates a decrease in miRNA expression in infected patient serum relative to that of uninfected patients, while positive change signifies the converse.

10.4 AOVET

Fatigue life assessment of two hybrid plate designs for canine pancarpal arthrodesis (PancarFat) (I Zderic, P Varga, B Gueorguiev)

Background: Hybrid locking canine pancarpal arthrodesis (PCA) plates with an oval (OH) instead of a round (RH) hole for the radiocarpal (RC) screw insertion would improve versatility in surgical application, however, may be more prone to failure due to the enlarged hole dimension.
Goal: To investigate mechanically the fatigue life the PCA plates with RH and OH design.
Results: Ten PCA plates with RH design and twenty PCA plates with OH design were assigned to 3 groups, pre-bent at 20° and fixed to a canine forelimb model with simulated distal radius, RC bone and third metacarpal bone. The OH plates were instrumented with an RC screw inserted either most proximally (OH-P) or most distally (OH-D) in the plate hole. All specimens were cyclically tested to failure at 8 Hz under 320 N. Cycles to failure were higher in the group with RH plate fixation versus both OH-P and OH-D plate configurations, reaching significance versus OH-D. No significant difference was detected between the OH-P and OH-D groups.

Despite the surgical advantages of the PCA plate with an oval RC screw hole, its fatigue life is significantly shorter versus the plate design with a round RC screw hole and therefore mitigates the potential benefits. The failure probability of the plate with an oval RC hole is increased regardless from the screw position in this hole. The plate with an oval RC screw hole cannot be recommended for clinical use.

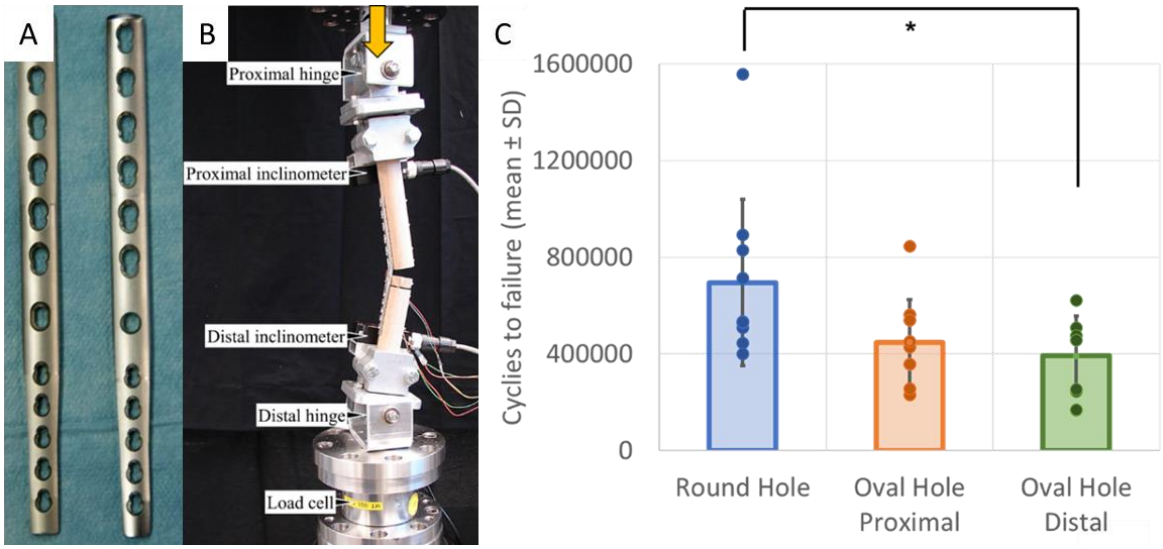


Figure 10.4.1: Fatigue life assessment of the two hybrid plate designs for canine pancarpal arthrodesis: A) 12-hole hybrid pancarpal arthrodesis plate with an oval (left) or a round (right) radiocarpal hole; B) Setup with a specimen mounted for mechanical testing; C) Cycles to failure in the three study groups with a star indicating significant difference.

Partners:

- Déjardin LM (Prof), Michigan State University, East Lansing, MI, USA
- Kowaleski M (Prof), Tufts University, Medford, MA, USA
- Asimus E (Prof), Ecole Nationale Vétérinaire de Toulouse, Toulouse, France
- Boudrieau RJ (Prof), Tufts University, Medford, MA, USA
- Saunders B (Prof), Texas A&M University, College Station, TX, USA
- Drenchev L (Prof), Bulgarian Academy of Sciences, Institute of Metal Science 'Acad. A. Balevski', Bulgaria

10.5 AOTC System

Statistical analysis of complex proximal humerus fractures (HumFx) (Ongoing) (P Varga, K Mys, A Bashardoust, T Pastor, J Dauwe, D Gehweiler, B Gueorguiev)

Background: Fixation of complex proximal humerus fractures remains challenging, partially due to the large variation in the number, shape and dislocation of fragments. Understanding the variability of fracture patterns could aid surgical training and education and contribute to implants development.
Goal: To perform statistical description of the pattern and investigate the spatial distribution of complex fractures at the proximal humerus.
Results: Anonymized preoperative CT datasets of 50 patients with three- or four-part proximal humerus fractures were collected retrospectively. The fracture fragments were identified semi-automatically on the CT datasets using advanced custom-developed image processing tools. The fractures were virtually reduced by identifying their fracture lines and solving the 3D puzzling problem utilizing the intact contralateral side as template.

A statistical shape model of the proximal humerus was built for this cohort and the variability in fracture patterns is described by projecting the fracture lines on the averaged bone surface and evaluating their spatial distribution.

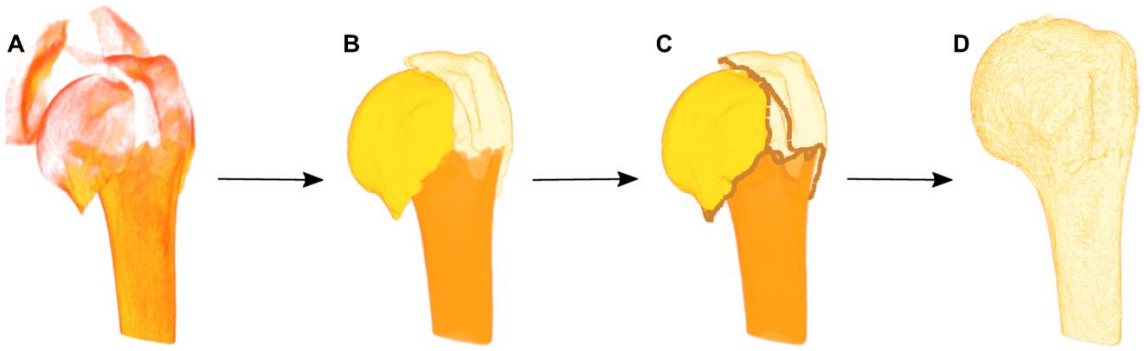


Figure 10.5.1: Workflow of virtual fracture reduction. The CT image (A) is segmented to recognize the bone fragments (B) and identify the corresponding fracture lines (C) that are used to solve the 3D puzzling problem and achieve virtual fracture reduction (D).

Pres:
Dauwe J, Mys K, Putzeys G, Schader JF, Richards RG, Gueorguiev B, Varga P, Nijs S. Three-dimensional segmented CT images help orthopedic surgeons and residents to correctly classify proximal humeral fractures. 2020 DKOU (oral).

Pub:
Dauwe J, Mys K, Putzeys G, Schader JF, Richards RG, Gueorguiev B, Varga P, Nijs S. Advanced CT visualization improves the accuracy of orthopaedic trauma surgeons and residents in classifying proximal humeral fractures: a feasibility study. Eur J Trauma Emerg Surg. 2020;epub.

Partners:
- Knobe M (Prof), Kantonsspital Luzern, Switzerland
- Lambert S (MD), University College London Hospital, UK
- Nijs S (Prof), UZ Leuven, Belguim

Biomechanical investigation of intrathoracic versus extrathoracic rib fracture plating (RibPlate) (D Mischler, C Schopper, B Gueorguiev)

Background: The high morbidity following surgical interventions on the chest wall due to large incisions, especially when treating subscapular fractures, often prevents surgeons from operative rib fracture fixation. Minimally invasive approaches to the intrathoracic side of the rib could allow for smaller incisions with lower morbidity, while maintaining construct stability. The biomechanical competence of intrathoracic versus extrathoracic rib plating was investigated together with the biomechanical performance of two versus three screws per fracture fragment in a previous phase of this project with the use of forty paired ribs.
Goal: To investigate (1) whether the cortical bone mineral density (BMD) and cortical thickness of the previous biomechanically tested ribs have acted as confounding factors on those results and (2) whether the clinical CT (QCT) resolution is sufficient for use in future finite element (FE) simulations of plated ribs.
Results: The CT data of the forty paired ribs, scanned with QCT, was converted to BMD values using a density phantom. The cortical and trabecular bone regions of each rib were separated and the cortical BMD was determined by averaging all corresponding values. The cortical thickness was calculated by a sphere fitting algorithm in the cortical bone region.

Both, cortical BMD and cortical thickness were not significantly different between the previous biomechanically tested groups, suggesting that these two parameters did not act as confounding factors in the study. Further, the QCTs of some ribs were compared with their high-resolution peripheral quantitative CTs (XCTs). Substantial differences between the two scan types were demonstrated, suggesting that the QCT resolution might be too low for use in reliable FE simulations.

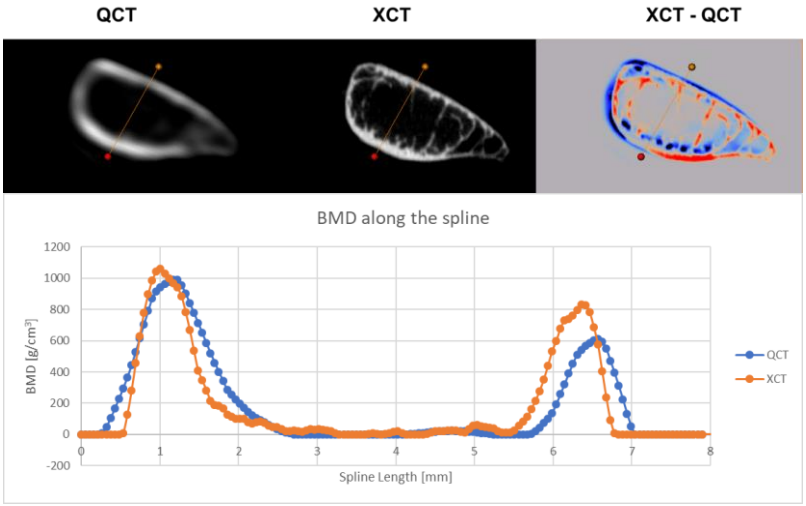


Figure 10.5.2: Comparison between QCT (top, left) and XCT (top, centre) scans. The processed difference between the scans (top, right) demonstrates substantial local changes in BMD, with either XCT (red) or QCT (blue) domination. By plotting the BMD along a spline (top, red line), the differences are pronounced in the cortex with QCT exhibiting lower BMD peak with a wider base around it. This blurring effect is likely due to the lower QCT resolution.

Pres:
Gueorguiev B, Mischler D, Schopper C, Schulz-Drost S, Brace M, Gaspari M. Is intrathoracic rib plate fixation advantageous over extrathoracic plating? 2020 DKOU (oral).
Mischler D, Schopper C, Gaspari M, Schulz-Drost S, Brace M, Gueorguiev B. Biomechanical human cadaveric investigation of intrathoracic versus extrathoracic rib fracture plating. 2020 DKOU (oral).

Partners:
- Gasparri M (Prof), Medical College of Wisconsin, USA
- Schulz-Drost S (MD), University Hospital Erlangen, Germany
- Brace M, DePuy Synthes, USA

BacterioPHAGE therapy for fracture-related infection due to Staphylococcus aureus (PhageS) (Ongoing) (F Moriarty, V Post, S Zeiter)

Background: The use of medical devices such as fracture fixation devices has had an enormously positive impact on patient care. However, approximately 5% of patients, across all medical specialties, can develop an infection associated with the device, which can have disastrous consequences. These bacterial infections involve biofilm formation and are therefore always highly antibiotic tolerant, even in the absence of specific antibiotic resistance genes. The antibiotic recalcitrance of the biofilm leads to poor treatment success rates and often requires implant removal to treat the infection.
Goal: The project aims to provide proof of concept data for the treatment of complicated Fracture-related Infection (FRI) with bacteriophage applied in buffered solutions or in a hydrogel formulation. For the first series of studies, we tested the efficacy of a phage-loaded thermo-responsive hyaluronan gel to prevent FRI in a rabbit model.
Results: In the prevention group, infection was established by inoculation with a clinical isolate of *S. aureus* whereafter the surgical site was exposed to either 1) 5 mL of phage in suspension (10^8 PFU/ml in phage buffer) or 2) phage-loaded hydrogel. After surgery, the animals were observed for one week after which they were euthanized. No antibiotics were administered postoperatively. Results are shown in the Figure 10.5.3.

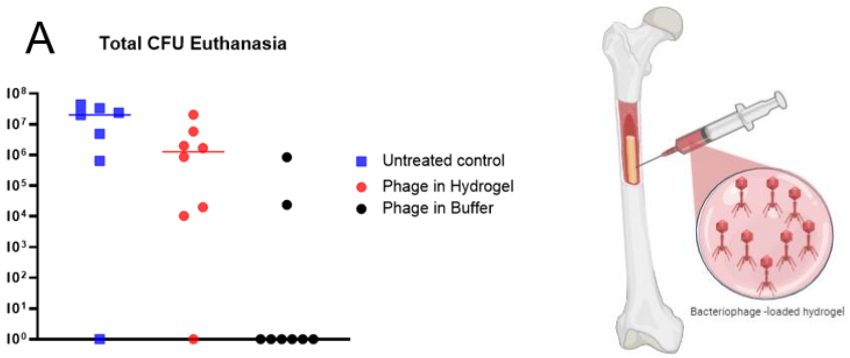


*Figure 10.5.3: Quantitative bacteriological evaluation is shown for control, phage-loaded hydrogel and phage in buffer treated animals receiving treatment **A:** soon after inoculation as a prevention measure. Note phage in buffer (black dots) are the best performing intervention in this study.*

Bacteriophage have significant effect in preventing infection, and this is most effective when given in buffer, which presumably allows more rapid distribution of phages in the early postoperative phase. In contrast, bacteriophage may have significant effect in treating infection when given in the hydrogel, which allows continuous phage release and distribution of phage over a longer period.

Pub:
J Onsea, J Wagemans, JP Pirnay, M Di Luca, M Gonzalez-Moreno, R Lavigne, A Trampuz, TF Moriarty, WJ Metsemakers. *Bacteriophage therapy as a treatment strategy for orthopaedic device-related infections: where do we stand?* Journal: eCM; Type of publication: Review.

Partners:
- Lavigne R, KU Leuven Belgium
- Trampuz A (MD), Charite Berlin, Germany
- Metsemakers WJ (MD), KU Leuven, Belgium

10.6 ARI Exploratory Research

Feasibility of the AO Fracture Monitor for measuring spinal fusion (SmartFusion) (V Varjas, M Windolf)

Background: CT-based monitoring of spinal fusion cases has multiple limitations. Beside the radiation exposure to the patient, the interpretation of CT images is highly subjective. Furthermore, CT only provides a coarse visual overview (snapshots) of the fusion process as no continuous mechanical data is available. Implantable sensor devices such as the AO Fracture Monitor could provide a reliable and objective means to monitor the progress of fusion and rapidly react to complications such as implant loosening.
Goal: To investigate the feasibility of applying the AO Fracture Monitor measurement principle to spinal fusion cases.
Results: A rod-mounted sensor prototype was developed from the exiting fracture monitoring implant. One sheep was implanted with an asymmetric 3-level pedicle screw-and-rod configuration and sensors attached to the rods between the screw pairs. Two segments were treated for fusion with autologous bone grafting. The fusion progress was monitored over 16 weeks. After euthanasia, axial stiffness of both fused segments was biomechanically evaluated by static off-centric loading and was compared to a non-fused reference segment. The sensors worked seamlessly and transmitted continuous rod loading data in the course of the experiment. Sensor data progression was confirmed by the biomechanical test data suggesting that the AO Fracture Monitor measurement principle may also be applied to monitor spinal fusion cases. Further sheep will be operated to confirm these preliminary observations.

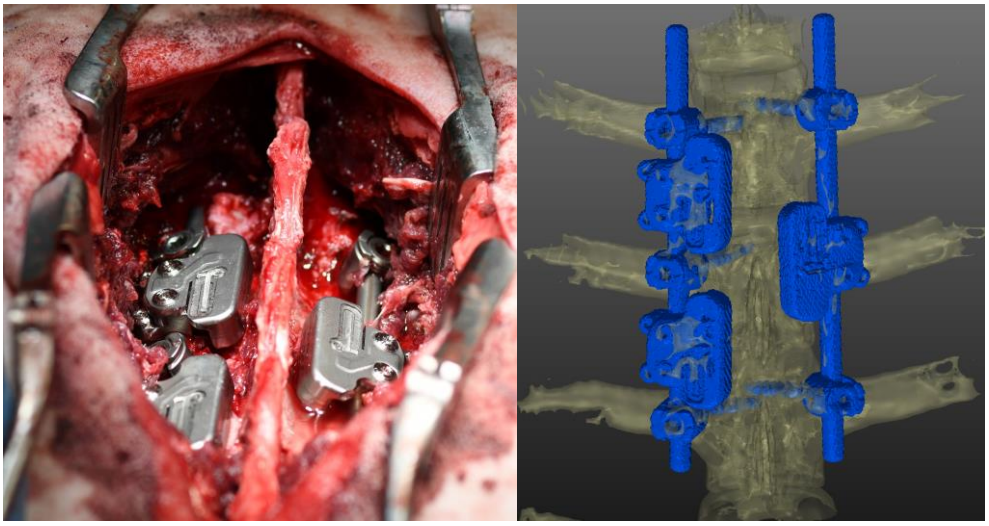


Figure 10.6.1: Left: instrumented spine of pilot sheep with three sensors attached to the pedicle screw rods. Right: post-op CT scan demonstrating the experimental configuration.

Partners:

- Benneker L (Prof), Inselspital Bern, Switzerland
- Davies E (MD), University Hospital Southampton, UK

A novel implant concept to amplify interfragmentary strain in large bone defects (StrainAmp) (Ongoing) (J Buschbaum, M Windolf)

Background: Bone defects resulting from trauma, infection and non-unions often require demanding treatment. Today, the defect can be either bridged with autologous bone graft to prevent secondary trauma, or it may be treated by bone lengthening or segment transport methods. In both cases the disturbing impact on the quality of life represents a significant burden to the patient. A novel implant concept may offer a quick one-step solution for critical-size bone defect healing. The concept intents to mechanically amplify the interfragmentary strain in a defect situation and optimize the biomechanical conditions. The underlying principle refers to Stephan Perren's strain theory, which implies that optimal interfragmentary strain triggers a chain of mechanobiological events inducing and enabling bone repair.
Goal: To develop a strain amplifying implant prototype for first *in vivo* application and to investigate the concept in a pilot preclinical experiment.
Results: Based on the strain amplification principle, an implant prototype suitable for *in vivo* use was developed and designed for use with the ARI's Quarter-Pipe dynamic fixator system for controlled axial stimulation. The device was implanted in two pilot sheep with a 30 mm transverse tibial defect to assess its ability to trigger and improve healing in large bone defects. The investigation is currently ongoing.

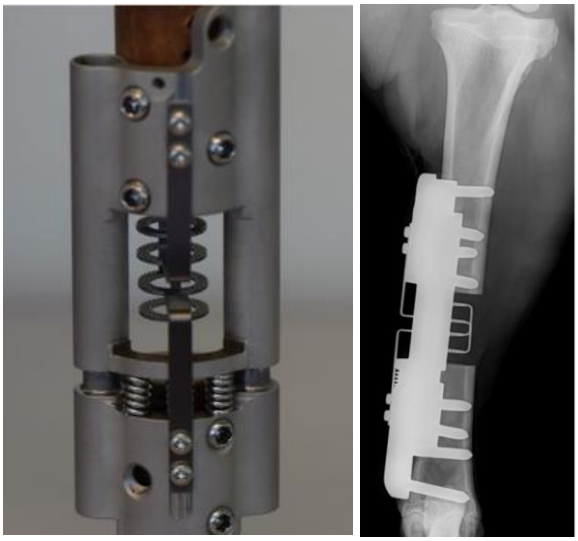


Figure 10.6.2: Strain amplifying implant with dynamic fixator (left) and post-operative radiograph of its application in a critical-size defect setting (right).

Partner:

- Perren N, Perrens 101 GmbH, Davos, Switzerland

Standardized fractures introducing a novel approach for orthopedic implant testing (J Schader, I Zderic, B Gueorguiev)

Background: Currently, there is no consensus on whether creation of standardized clinically relevant bone fractures or their simulation via osteotomizing result in more realistic outcomes with regard to biomechanical testing of orthopedic implants.
Goal: To set and analyze the biomechanical behavior of standardized stable pertrochanteric fractures versus their simulation via osteotomizing.
Results: Eight pairs of fresh-frozen human cadaveric femora were assigned in paired fashion to two study groups. In Group 1, stable pertrochanteric fractures AO/OTA 31-A1 were created via constant force application on the anterior cortex of the femur through a blunt guillotine blade. The same fracture type was simulated in Group 2 by means of osteotomies. Based on CT scans of the intact and fractured femora, a mean shape model was created to analyze the standardization of the fractures. All femora were implanted with a dynamic hip screw and

biomechanically tested in 20° adduction under progressively increasing cyclic axial loading at 2Hz. Femoral head fragment movements with respect to the shaft were monitored by motion tracking. Fracture lines of all eight fractured specimens were located within the pertrochanteric region of the proximal femur, including their mean and standard deviation (SD) representatives. From a biomechanical point of view, by resulting in more consistent outcomes under dynamic loading, standardized fracture creation may be more suitable for orthopedic implant testing as compared to osteotomizing.

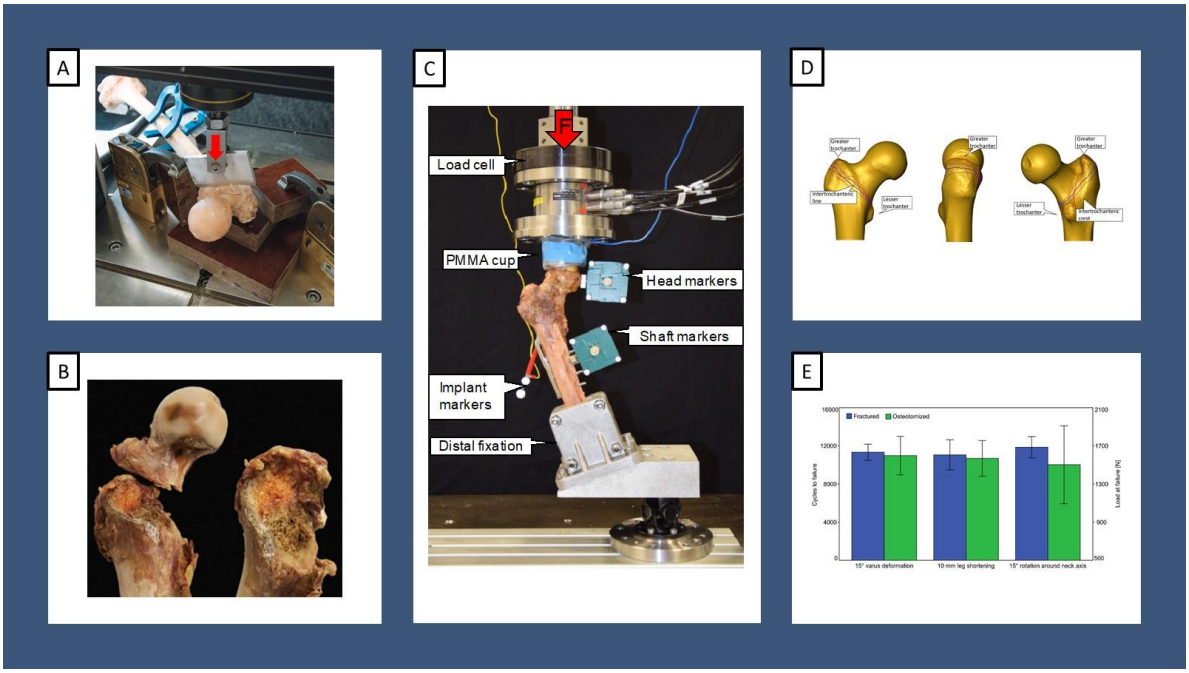


Figure 10.6.3: A) Setup with a specimen mounted for creation of a standardized stable pertrochanteric fracture by means of a blunt guillotine blade, with red arrow indicating force direction; B) Visualization of the created patterns of a pertrochanteric fracture AO/OTA 31–A1; C) Test setup with a specimen mounted for biomechanical testing; D) Mean shape model visualizations in anterior, lateral and posterior views with mean (thick lines) and SD (thin lines) fracture line representatives for standardized fractures (red) and osteotomies (blue); E) Diagram presenting cycles to failure and failure load in the two study groups with fractured and osteotomized specimens according to the clinically relevant criteria 15° varus deformation, 10 mm leg shortening and 15° femoral head rotation around the neck axis.

Pres:

Schader JF, Zderic I, Dauwe J, Mys K, Gehweiler D, Acklin Y, Gueorguiev B, Stoffel K. Biomechanical comparison of standardized sstable pertrochanteric fractures versus osteotomies for orthopaedic implant testing. 2020 Graubünden forscht (oral).

Schader JF, Zderic I, Gehweiler D, Dauwe J, Mys K, Danker K, Acklin Y, Gueorguiev B, Stoffel K. Standardized fractures – introducing a novel approach to orthopedic implant testing. 2020 EORS (poster).

Partners:

- Stoffel K (Prof), University Hospital Basel, Basel, Switzerland
- Acklin YP (MD), University Hospital Basel, Basel, Switzerland

Investigation of the pharmacokinetics of fentanyl patches at different locations on sheep (Fentasheep) (T Buchholz, S Zeiter)

Background: The sheep is a frequently used animal model for orthopedic research at the ARI mostly involving invasive surgery on the hind limb. Therefore, the sheep need general anesthesia. These painful procedures can only be ethically justified with the application of an adequate analgesia protocol. A transdermal fentanyl patch is a great way of application to administer fentanyl over a longer period avoiding stressful injections for the animal. The uptake of fentanyl through the skin depends on various factors (fat content of the skin, temperature, blood circulation). A comparison of different locations is needed to clarify the best and most feasible skin area to apply a transdermal fentanyl patch. With an identification of the characteristics of the uptake of fentanyl in sheep, the current analgesia protocol could be adapted in order to be more reliable.
Goal: In accordance with the refinement of the 3R principle, the aim of this study was to improve the analgesia protocols used at the ARI for sheep undergoing orthopedic surgery.
Results: The patch applied on the foreleg resulted in a faster fentanyl uptake with higher peaks and a longer time within or above the target fentanyl plasma concentration when compared to the one on the thorax. Additionally, it was easier to apply the patch at the foreleg than at the thorax. Our findings suggest that the fentanyl patch should be applied to the foreleg 3–6 h before the painful insult and that its effect should last at least 48 h.

Pub:
Buchholz T, Hildebrand M, Heider A, Stenger V, Arens D, Spadavecchia C, Zeiter S. Transdermal Fentanyl Uptake at Two Different Patch Locations in Swiss White Alpine Sheep. Animals (Basel). 2020 Sep 17;10(9):1675. doi: 10.3390/ani10091675. PMID: 32957484; PMCID: PMC7552603.
Thesis:
Buchholz Tim. Transdermal Fentanyl Uptake at Two Different Patch Locations in Swiss White Alpine Sheep. Dr med vet Thesis. University of Bern, 2019.

Partners:
- Spadavecchia C (Prof) VetSuisse University of Bern, Switzerland
- Rohrbach H (Dr med vet), VetSuisse University of Bern, Switzerland
- Heider A, Swiss Institute of Allergy and Asthma (SIAF), Davos, Switzerland

Establishing the interplay of soluble and physical signals on endochondral development (MechSignal) (Started) (A Armiento, M Stoddart)

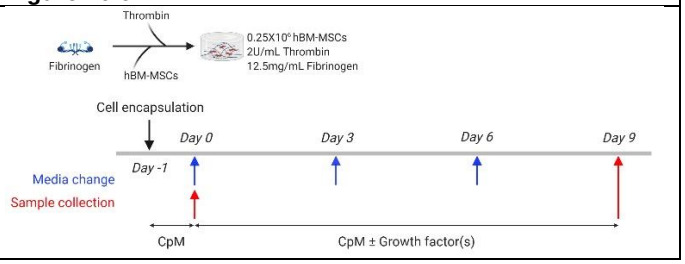
Background: Endochondral and intramembranous ossification describe the two natural healing mechanisms of long bone fractures, during which the locally applied strain is known to affect the final healing outcome. Locally-derived progenitors from bone marrow and periosteum are major cellular contributors to the fracture callus, being able to undergo endochondral and intramembranous differentiation to differing degrees. While controlling the mechanical environment across a fracture gap is a well-accepted, classical AO principle, there is little understanding of the underlying biological changes. A greater understanding of the underlying biological responses to the soluble and physical factors present during early healing, coupled with potential markers related to mechanical stimulation, may help in assessing at early time points whether a fixation is suitably stable for the desired healing outcome.
Goal: This project aims to create an *in vitro* model to study the interplay between signaling molecules such as TGF-β, BMPs and nerve growth factor (NGF), cell types including mesenchymal cells and macrophages, and mechanical load. This model will uncover cellular/molecular aspects of endochondral differentiation during fracture healing.
Results: Cell-laden fibrin gels are cultured in serum-free chondro-permissive medium (CpM) for 24h. The samples are then divided in sixteen different groups based on the growth factor supplementation (Figure 10.6.4A) and cultured for nine days. Medium supplemented with growth factors is refreshed every third day and samples are collected at day 0 and day 9

(Figure 10.6.4B). Within the first 24h the fibrin gels remodel into 3D pellets with an average diameter of 1 mm and 1.5 mm when in absence or presence of TGF-β1, respectively. The pellet size positively correlates with the DNA content. sGAG production is sustained by TGF-β1 supplementation and enhanced when in combination with NGF and BMP-2. The secretion of alkaline phosphatase is driven by the combined effect of TGF-β1 and BMP-2. Gene expression analysis shows an overall trend for cartilage matrix protein upregulation while the enzymes associated with matrix degradation are downregulated.

Figure 10.6.4A

	ng/mL			
Group	TGFB1	BMP2	BMP9	NGF
1	0	0	0	0
2	10	0	0	0
3	0	100	0	0
4	0	0	1	0
5	0	0	0	50
6	10	100	0	0
7	10	0	1	0
8	10	0	0	50
9	0	100	1	0
10	0	100	0	50
11	0	0	1	50
12	10	100	1	0
13	10	100	0	50
14	10	0	1	50
15	0	100	1	50
16	10	100	1	50

Figure 10.6.4B



Pub:

Angela Rita Armiento, Luan Phelipe Hatt, Guillermo Sanchez Rosenberg, Keith Thompson, Martin James Stoddart. Functional Biomaterials for Bone Regeneration: A Lesson in Complex Biology. *Adv. Funct. Mater.* 2020 Feb; 1909874.

Identification of mechanical conditions promoting hypertrophic endochondral differentiation *in vitro* (MechEndro) (Started) (S Verrier, M Stoddart)

Background: It is widely accepted that secondary fracture healing requires a certain level of mechanical stimulation to initiate and promote callus formation. By means of *in vivo* experimental setups, several groups have shown that cyclic compressive strain applied to a diaphyseal fracture fosters healing via the formation of a stronger cartilaginous callus leading to earlier bone bridging. Though, optimal loading parameters have not yet been entirely defined. There are still uncertainties concerning the magnitude of strain, its frequency, optimal temporal distribution and duration. More importantly, the influence of those parameters on the cellular process of hypertrophic cartilage formation and remodeling - critical for bone healing - is still not fully understood.

Goal: To better understand the biological effect of strain at the cellular level. We specifically aim to define *in vitro* the lower strain induction limit for the hypertrophic endochondral differentiation of MSC and callus-like matrix formation.

Results: For this project, a customized multi-well bioreactor system was developed. The device enables uni-axial defined deformation of 24 tissue engineered constructs in parallel and offers large flexibility in the choice of both strain level to be applied, and application of multi-segmental deformation protocols. In preliminary studies aiming to validate the function and reliability of the bioreactor, mesenchymal stem cells (MSCs) were seeded in 2% agarose gels as well-defined model material. Samples were cultured under different mechanical loading conditions (0, 10 and 30% strain) either in presence of classical chondrogenic medium (containing 10 ng/mL transforming growth factor β1 (TGFβ1)), or chondro-permissive medium, in which TGFβ1 was omitted. Over three weeks of continuous cyclic deformation, no adverse events were experienced. Cell viability remained comparable between all conditions, and agarose constructs remained intact even when deformed by 30% of their height. Real time PCR analysis also showed regulation of specific genes, such as Cartilage oligomeric matrix protein (COMP) or Matrix metalloproteinase (MMP)-13 in response to the strain magnitude, indicating an effective transmission of the deformation to the model agarose constructs, and a response of the cells to strain.

Partners:

- RISystem AG, Landquart, Switzerland
- Perren N, Perrens 101 GmbH, Davos, Switzerland

Anti-inflammatory therapy for cartilage preservation (CartRegen) (Started) (Z Li, S Grad)

Background: Osteoarthritis (OA) affects a large proportion of the population and is associated with significant burden on patients and health care systems. Traumatic joint injury is a major risk factor for the development of OA. Early intervention after an acute injury may therefore prevent progressive joint deterioration. It is believed that the acute inflammatory response plays a major role in the progression towards the chronic painful condition. Early anti-inflammatory and chondroprotective treatment may halt or even reverse the deteriorating process.
Goal: The aim of this project is to evaluate the chondroprotective and anti-inflammatory activity of small molecule compounds in an inflammatory 3D pellet culture system and cartilage injury explant model under bioreactor controlled mechanical load. The overall goal is to generate pre-clinical data and identify small molecules for early intervention and prevention of post-traumatic osteoarthritis.
Results: Six small molecules (RCGD-423, Wogonin, XAV-939, Zingerone, THSG, and Paeonol) were evaluated biochemically and histologically for their anabolic and anti-inflammation effects on 3D pellets of human OA chondrocytes. 3D pellets of human OA chondrocytes were cultured in chondrogenic medium for 7 days to generate cartilage tissue followed by small molecule treatment for 3 days within chondropermissive medium supplemented with 1 ng/mL IL-1β + 1 ng/mL TNF-α.
Compared with IL-1β+TNF-α group, Zingerone and Paeonol down-regulated MMP3 gene expression. Paeonol, XAV-939, THSG, and Wogonin up-regulated COL2 and ACAN gene expression. Wogonin, XAV, RCGD-423, Zingerone, and THSG down-regulated expression ratio of inflammatory/anti-inflammatory genes IL6/IL10 and IL8/IL10. At protein level, Zingerone, Wogonin, and XAV-939 down-regulated IL-8 expression; RCGD-423 up-regulated IL-10 expression. Compared with the pellets cultured in chondrogenic medium, pellets from the inflammatory model group did not show proteoglycan staining of extracellular matrix. Paeonol, Wogonin and XAV-939 enhanced proteoglycan accumulation in pellets (Figure 10.6.5). Summarizing all the factors evaluated *in vitro*, XAV-939 and Wogonin showed the most pronounced anti-inflammatory and regenerative effect, as indicated by the highest numbers of positive outcome variables.

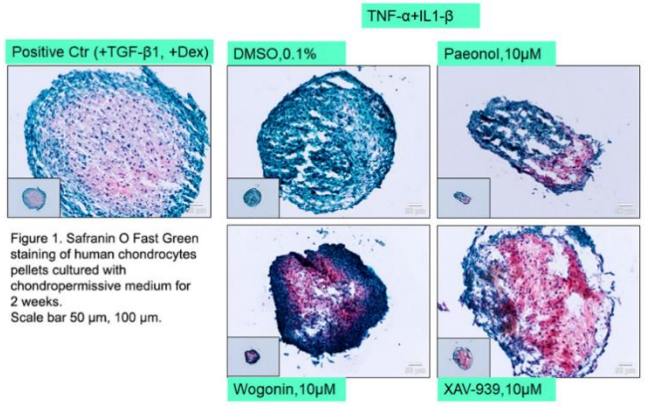


Figure 10.6.5: Safranin O Fast Green staining of human chondrocytes pellets cultured with chondropermissive medium for 2 weeks. Scale bar 50 μm, 100 μm.

Pres:
Zhang P, Basoli V, Ziadlou R, Grad S, Li Z. *In vitro* and *ex vivo* models to test the anti-inflammatory and regenerative effects of small molecules in osteoarthritis. Graubünden Forscht, Davos, Sep 23-24, 2020 (poster).

Pub:
Vernengo AJ, Grad S, Eglin D, Alini M, Li Z. Bioprinting tissue analogues with decellularized extracellular matrix bioink for regeneration and tissue models of cartilage and intervertebral discs. Advanced Functional Materials. 2020; 30:1909044.

Partner:
- Xinluan Wang (Prof), Shenzhen Institute of Advanced Technology, Shenzhen, China

Optimized chondrogenesis in an osteochondral defect model (VariDon2) (Started) (M Stoddart, E Della Bella)

Background: Bone marrow derived stem (or stromal) cells (BMSCs) have been proposed a source of cells for autologous cell therapy. While showing promise *in vitro*, translation into the clinics has proven challenging. One reason for this is the inability to accurately predict cell function and hence, whether cells from a patient will behave in a predictable manner. In a previous AO funded study (Varidon), we defined a TGF-β receptor ratio that was predictive of chondrogenesis. Furthermore, by relatively simple manipulation of the receptor ratio we could convert non-responsive donors and make them responsive to chondrogenic signals.
Goal: Within this study, we aim to develop this technology further to improve chondrogenic differentiation within biomaterials with implant design in mind. Furthermore, we will activate chondrogenesis by way of multiaxial load, in an *ex vivo* endochondral defect model that more faithfully resembles a cartilage defect.
Results: Optimization of siRNA delivery into hydrogels is carried out with GelMA first, then the methods will be adapted for use in the other candidate hydrogels, such as fibrin, alginate IPN and THA-collagen. 10 pmol of *TGFBR2* or negative-siRNAs have been loaded into Fuse-It-siRNA liposomal carrier, that were integrated into cell-laden GelMA gels (50% DS, 8% gel, 20x10^6 cells/ml). The constructs were maintained in culture with chondropermissive medium. The Live/Dead staining showed high cell viability up to 7 days and downregulation of *TGFBR2* after 48 hours from construct assembly (Figure 10.6.6, preliminary data from two donors).

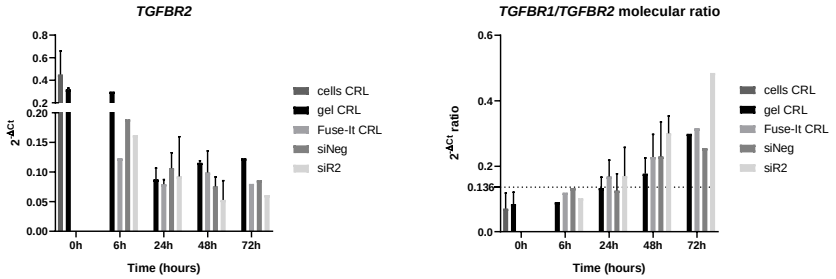


Figure 10.6.6 – Expression of TGFBR2 and TGFBR1/R2 ratio following siRNA treatment.

Sorting based on TGF-βRI and TGF-βRII surface expression was tested with two MSC donors (Figure 10.6.7).

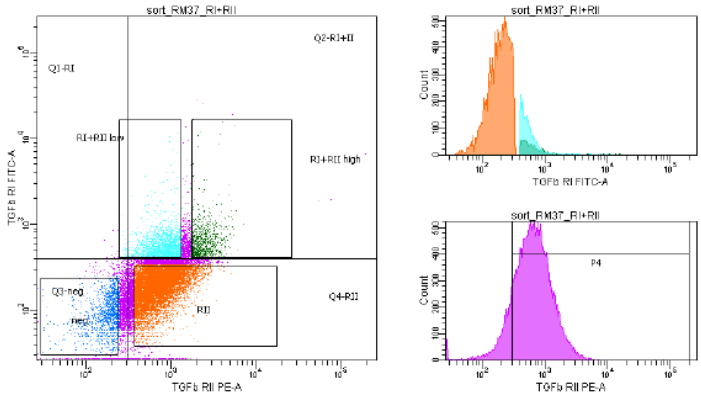


Figure 10.6.7 - Sorting strategy to separate populations of BMSCs based on TGF-βRI and TGF-βRII expression. The populations are divided into $R1^{neg}R2^{neg}$, $R1^{neg}R2^{pos}$, $R1^{pos}R2^{dim}$, $R1^{pos}R2^{bright}$.

Pub:
Jahangir S, Eglin D, Pötter N, Khozaei Ravari M, Stoddart MJ, Samadikuchaksaraei A, Alini M, Baghaban Eslaminejad M, Safa M. Inhibition of hypertrophy and improving chondrocyte differentiation by MMP-13 inhibitor small molecule encapsulated in alginate-chondroitin sulfate-platelet lysate hydrogel. Stem Cell Res Ther. 2020 Oct 9;11(1):436. doi: 10.1186/s13287-020-01930-1. PMID: 33036643.

Local delivery of IL-1Ra as a strategy to enhance long bone healing (HealBone) (Ongoing) (W Lackington, K Thompson)

Background: Although the majority of patients with bone fractures typically heal without complications, a small proportion (up to 10%) of patients display healing deficiencies. In such patients, there appears to be an inappropriately maintained pro-inflammatory environment throughout the healing process that acts to diminish healing capacity. Therefore, anti-inflammatory strategies to target the local fracture microenvironment may be an effective way to improve fracture healing. This project focuses on a specific anti-inflammatory protein, (interleukin-1 receptor antagonist) IL-1Ra, the receptor antagonist of the potent pro-inflammatory cytokine IL-1β.
Goal: This project seeks to investigate the efficacy of strategies to antagonize IL-1β-driven inflammation to promote bone healing, and also to determine whether such a strategy may enhance BMP-2-mediated bone formation, in challenging fracture healing environments.
Results: A non-viral gene delivery system was assessed *in vitro* to determine the efficacy of IL-1Ra delivery for antagonizing the inhibitory effect of IL-1β on osteogenic differentiation of rat mesenchymal stromal cells (MSCs). Gene delivery of IL-1Ra (mediated via polyethylenimine (PEI) as a DNA carrier) could effectively block the negative effects of IL-1β on rat MSC osteogenesis (Figure 10.6.8). We are currently in the process of confirming whether local delivery of IL-1Ra has synergistic effects when delivered in combination with sub-optimal doses of BMP-2 in a rodent femoral defect model in skeletally mature female Fischer 344 rats using internal plate fixation.

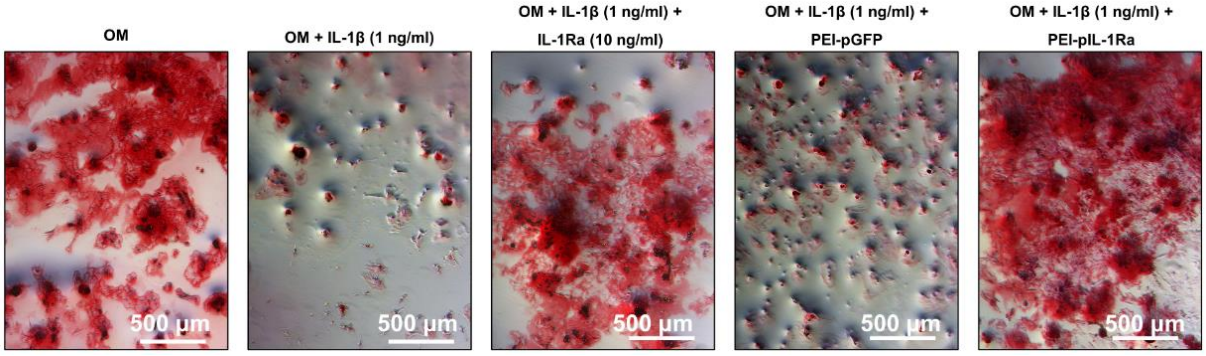


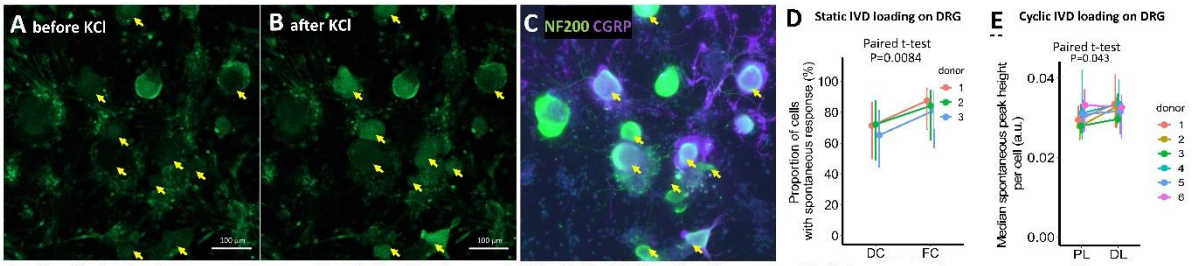
Figure 10.6.8: Non-viral gene delivery of IL-1Ra overcomes the inhibitory effect of IL-1β on osteogenic differentiation of rat MSCs, as assessed by Alizarin red staining. PEI = polyethylenimine.

Pub:

Lackington WA, et al., Non-viral Gene Delivery of Interleukin-1 Receptor Antagonist Using Collagen-Hydroxyapatite Scaffold Protects Rat BM-MSCs From IL-1β-Mediated Inhibition of Osteogenesis. *Front. Bioeng. Biotechnol.* (2020) 8:1180.

Link mechanics, degeneration and discogenic pain (LINK) (Ongoing) (M Peroglio, J Ma, S Grad, M Alini)

To study the mechanism of low back pain, how detrimental loading on intervertebral disc (IVD) influences peripheral nerve sensitization (dorsal root ganglion, DRG) was investigated. Static loading causing IVD narrowing was compared with the loading maintaining the initial height. Cyclic loading with high or normal intensity and frequency was also compared. The conditioned medium was applied to adult bovine DRG neuron culture. Spontaneous calcium oscillation in the neurofilament 200 (NF200) expressing mechanoreceptors was slightly elevated under the influence of static IVD-narrowing loading (Figure D) and cyclic loading with high intensity and frequency (Figure E), which indicates pain related neuronal discharge.



A and B: calcium imaging of neurons, response to potassium chloride validate the neuronal viability (viable cells indicated by yellow arrow). C: Immunofluorescent labelling of DRG neuronal subpopulation when analyzing the calcium imaging results.

DC: displacement control
FC: force control (IVD narrowing)

PL: normal frequency intensity
DL: high frequency intensity

Pres:

Gewiess J, Ma J, Grad S, Alini M, Richards RG, Peroglio M. Influence of constant intervertebral disc compression on pain-related nerve sensitization. 'Graubünden Forscht 2020 Poster presentation'. 23-24 Sep 2020. Online virtual meeting.

Ma J, Soubrier A, Grad S, Alini M, Peroglio M. Detrimental Mechanical Loading of Intervertebral Discs Promotes Sensitization of Dorsal Root Ganglion Neurons. '2020 World Congress on Pain Poster presentation'. postponed to June 2021. Amsterdam, The Netherlands.

Ma J, Patil V, Pandit A, L Quinlan, DP Finn, Grad S, Alini M, Peroglio M. Inflammatory Cytokines Sensitize Dorsal Root Ganglion Neurons and Activate Spinal Cord Glia. '2020 World Congress on Pain Poster presentation'. postponed to June 2021. Amsterdam, The Netherlands.

Ma J, Hildebrand M, Basoli V, Grad S, Alini M, Peroglio M. Predictability of dorsal root ganglion neuronal response to hypoxic stress: a cell line, primary cell and organ model validation study. '11th World Congress on Alternatives and Animal Use'. postponed to 22-26 August 2021. Maastricht, The Netherlands.

Wangler S, Menzel U, Li Z, Ma J, Hoppe S, Benneker LM, Alini M, Grad S, Peroglio M. Whole organ cultures as advanced models to assess stem cell regenerative potential in degenerative disc disease. '11th World Congress on Alternatives and Animal Use'. postponed to 22-26 August 2021. Maastricht, The Netherlands.

Pub:

Ma J, Stefanoska D, Grad S, Alini M, Peroglio M. Direct and Intervertebral Disc Mediated Sensitization of Dorsal Root Ganglion Neurons by Hypoxia and Low pH. Neurospine. 2020 Mar;17(1):42-59.

Ma J, Stefanoska D, Stone LS, Hildebrand M, Donkelaar CC, Zou X, Basoli V, Grad S, Alini M, Peroglio M. Hypoxic Stress Enhances Extension and Branching of Dorsal Root Ganglion Neuronal Outgrowth. JOR spine. 2020;e1090

Automation of time-lapse analyses (AUTO, Feasibility study) (M Peroglio, A Soubrier, K Mys, M Junxuan)

Background: Calcium imaging methods based on influx of fluorescent-labelled calcium ion binding dyes are commonly used to investigate neural sensitization. These dyes allow both spontaneous and cytokine-induced neural firing to be monitored. In brief, neural cells are seeded on a plate, stimulated, and then evaluated using fluorescence calcium influx imaging methods. Following application of the dye, the fluorescent signal of the cells is followed over time (time lapse of 100-200 seconds). The extraction of numerical data from images involves many manual steps that is influenced by subjective judgement. This makes it not only very time-consuming, but also user-dependent variation is an issue.

Goal: Automate the quantification of time-lapse calcium signal in soma and neural cell outgrowth and hence establish a fast and user-independent analysis.

Results: Matlab software was used for complete workflow automation, including cell soma and outgrowth segmentation and quantification (size, mean gray value) (Figure 10.6.9).

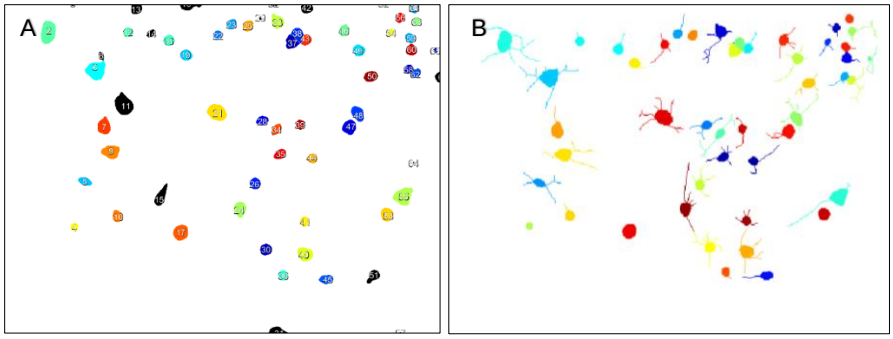


Figure 10.6.9: Example of a region of interest of an imaged well. A. Segmentation of soma automatically; B. Segmentation of outgrowth automatically.

Image analysis was compared between ImageJ, a standard image analysis package, and the developed Matlab algorithm. A similar segmentation was obtained using the 2 methods (Figure 10.6.10).

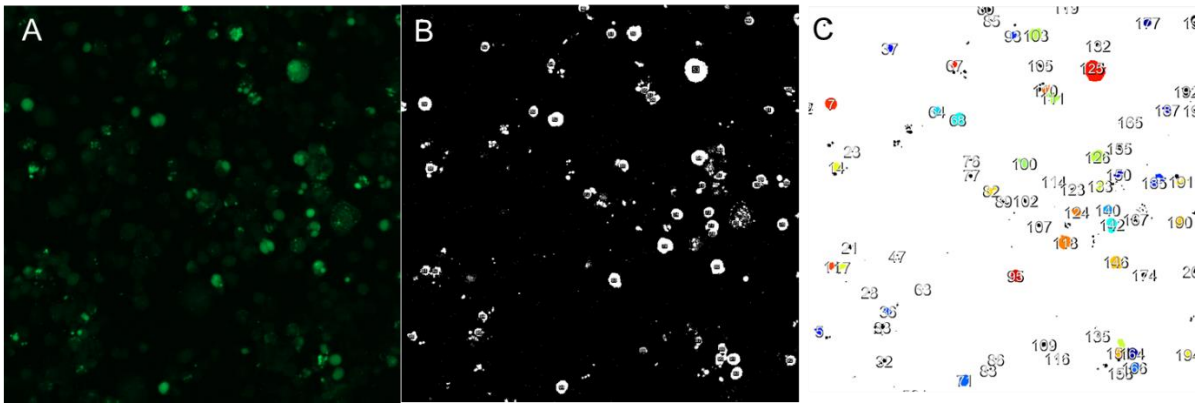


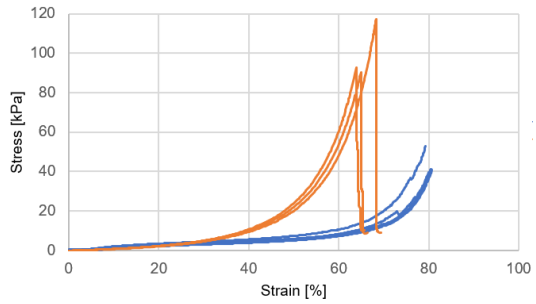
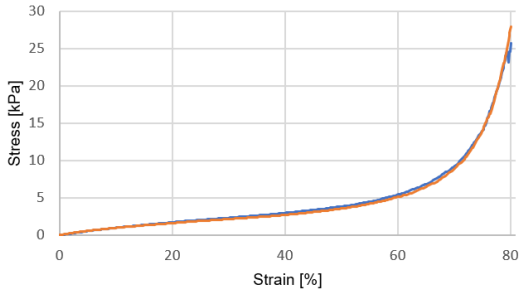
Figure 10.6.10: Comparison of ImageJ and Matlab for soma region of interest segmentation. A. The original image; B. Segmentation performed using ImageJ; C. Segmentation performed using Matlab.

The image analysis using the Matlab algorithm is completely automated, the user interaction is only needed to specify the input and output path. The user interaction that was around 1 hour/sample when using ImageJ is now reduced to around 5 minutes/sample.

Enhancing cartilage self-repair using cell-free IPN biopolymer hydrogels (GELHOME 2) (Ongoing) (M D'Este, D Eglin)

Background: Acute cartilage defects are a significant source of suffering and disability, leading to productivity loss and significant healthcare costs. Aging population and increase in physical activity at all ages are amplifying the societal impact of cartilage defects, which in the long-term contribute to osteoarthritis onset. Materials trying to match cartilage resilience are usually unsuitable for cell invasion, new tissue formation and adhesion to native tissue, which is the first requirement for lateral integration. Double-network hydrogels are specialized interpenetrating polymeric networks with outstanding strength and toughness. Double networks were initially developed from non-biodegradable materials unsuitable for long-term implantation. Recent advances have demonstrated how the same design paradigm can be employed to fabricate biopolymer-based tough double network hydrogels.
Goal: In this project, we are engineering combinations of naturally-derived biopolymers for obtaining hydrogels with high resilience and high strength to withstand repeated mechanical load, cytocompatibility and capability of being invaded by cells.
Results: Single network hydrogel polymers with complementary properties were combined into double networks through different approaches. Employing the inverted double network synthesis approach, the goal initially set of reaching compressive moduli and stresses at break

in the MPa order of magnitude was achieved using a composite from gelatin methacryloyl and collagen covalently crosslinked with glutaraldehyde. Overall, the main limitation was the capability of the second network to penetrate into the first one. Therefore, alternative approaches were investigated, including induction of macroporosity with controlled ice crystals formation and the use of electrophoresis to drive the diffusion of the second network.

Figure 10.6.11: characterization of cryogels	
	
Compression test of the same gelatin-based gel prepared with and without cryogelation, showing the increased modulus achieved with cryogelation.	*Cryogels can be compressed to 20% of their original height and preserve their mechanical properties upon repeated loading.*

Cryogel properties (Figure 10.6.11) make them good candidates to be employed as biodegradable scaffolds for bioreactor studies in the Regenerative Orthopedics program. Preliminary tests on electrophoresis also proved promising, and worth investigating further. Standard synthesis and the molecular stent method achieved inferior results in terms of homogeneity and improvement of mechanical properties compared to single networks.

Dissertation:
Taiyo Yamamoto**,** Synthesis of a double-network tough hydrogel from extracellular matrix derived polymers; Msc ETH Heath Sciences and Technology, Supervisor: Prof Matteo D'Este; Tutor: Prof Stephen J Ferguson.

Pub:
A Vernengo, Z Li, K Mys, P Varga, S Grad, M Alini, D Eglin, Annulus fibrosus tissue engineering using 3D printed poly(ε-caprolactone) scaffolds with oriented ply structure. , ORS, Phoenix AZ, 2020.
A Augurio, P Cortelletti, R Tognato, A Rios, R Levato, J Malda, M Alini, D Eglin, G Giancane, A Speghini, T Serra, A Multifunctional Nanocomposite Hydrogel for Endoscopic Tracking and Manipulation, Advanced Intelligent Systems 2(3) (2020) 1900105.
AHJ Gowda, Y Bu, O Kudina, VK Kanala, RA Bohara, D Eglin, A Pandit, Design of tunable gelatin-dopamine based bioadhesives, Int J Biol Macromol 164 (2020) 1384-1391.
BO Okesola, S Ni, B Derkus, CC Galeano, A Hasan, Y Wu, J Ramis, L Buttery, JI Dawson, M D'Este, ROC Oreffo, D Eglin, H Sun, A Mata, Growth-Factor Free Multicomponent Nanocomposite Hydrogels That Stimulate Bone Formation, Adv Funct Mater 30(14) (2020) 1906205.
P Behrendt, Y Ladner, MJ Stoddart, S Lippross, M Alini, D Eglin, AR Armiento, Articular Joint-Simulating Mechanical Load Activates Endogenous TGF-beta in a Highly Cellularized Bioadhesive Hydrogel for Cartilage Repair, Am J Sports Med 48(1) (2020) 210-221.

Partners:
- Ferguson S (Prof), ETH Zurich, Switzerland

10.7 OCD Consortium

3D printed constructs for osteochondral defect repair (OCD Consortium)

Osteochondral defects are still a major clinical challenge. They represent a large societal burden as they limit employment and impede daily life activities of millions of Europeans. Moreover, these injuries often lead to further degeneration of the joint, into a disabling disease known as osteoarthritis (OA). The defect bridges two major tissue types (cartilage and bone) that also have zonal structures within and specific healing capacities. Additionally, the cartilaginous surface must follow the patient specific contour of the surrounding tissue to avoid arthritic changes. The ARI collaborative research program (CRP) OsteoChondral Defect (OCD) brings together multidisciplinary expertise in materials, bioprinting, bioreactors, biomechanics, macrophages and animal models. Additive manufacturing and Biofabrication approaches are used to produce constructs systematically evaluated to assess the influence of physical and chemical parameters on cartilage and bone repair. In addition, as the immune response and inflammatory environment is known to directly influence the repair tissue produced, the effect of the material combination on macrophage behavior is being investigated. Bioreactor and culture models that include multiple tissues of the joint completed with immune cells are used to reduce *in vivo* experimentation along 3R Principles. Clinical insights drive the research of the OCD to ensure that a route to translation is always a consideration. Therefore, as an underlying principle, increases in implant complexity will be justified by significant increases in implant function, thus ensuring sufficient biological benefit of additional regulatory requirements.
The project started in June 2017 and is funded for 4 years. This strong consortium is composed of five teams respectively from the University of Pennsylvania, United State, with Dr Jason Burdick, Dr Claudia Loebel and Dr Robert Mauck; The University Medical Center Utrecht, the Netherlands, with Florencia Abinzano, Dr Riccardo Levato and Prof Jos Malda; The University Medical Center Rotterdam, the Netherlands, with Tim Wesdorp, Dr Yvonne Bastiaansen-Jenniskens, Dr Roberto Narcisi and Prof Gerjo JVM van Osch; the Chinese University of Hong-Kong, Hong-Kong, with Dr Kevin Ho and Prof Ling Qin, and the ARI, Switzerland, with Dr Andrea Schwab, Dr Matteo D'Este, Dr David Eglin, Prof Martin Stoddart, Prof Mauro Alini and Prof Geoff Richards with multidisciplinary expertise in materials, bioprinting, bioreactors, biomechanics, cell biology and immunology. The team is supported by Prof Peter Angele and Prof Peter Van der Kraan acting as advisory experts.

Multiple, crosslinkable bio-inks for 3D microextrusion of tissue-like constructs and biodegradable thermoplastic elastomer for fuse deposition manufacturing (Multibio-Ink) (Ongoing) (A Schwab, D Eglin, M D'Este, M Stoddart)

Background: 3D bioprinting technology has been recently employed to induce microarchitectural organization of collagen fibrils within a continuous hyaluronan (HA) matrix. The shear induced alignment and thus the control of collagen fibrils in a composite hydrogel opens a wide range of applications. We demonstrated that the zonal orientation of collagen fibers in articular cartilage can be mimicked with this technique (Schwab et al 2020, Mater Today Bio). To achieve integrative defect repair, chondrocytes should be able to migrate into the implant and this implant should integrate with the surrounding tissue to ensure complete defect filling.
Goal: Assessing chondrocyte's capability of invading a chemokine-free hydrogel in an ex-vivo cartilage ring model; verify how variations in composition influence chondrocyte invasion.
Results: With the aim to develop a cell free implant, the chondral layer should promote the infiltration of chondrocytes from the surrounding tissue. To address this question cell migration of endogenous chondrocytes into THA, collagen (col) and THA-col was investigated *in vitro* using a cartilage ring model. After 21 days of *in vitro* culture, brightfield images showed the presence of chondrocytes in the defect area (Figure 10.7.1B). These cells were migrating out of the cartilage ring towards the biomaterial filled in the cartilage defect. While col hydrogel was mostly invaded by cells, less cells were present in THA-col and in THA chondrocytes were located mainly at the explant-biomaterial interface. A scoring of Safranin-O stainings (migration

index), evaluating the biomaterial-tissue integration, the presence of cells on the biomaterial periphery and within the biomaterial resulted in an enhanced migration of cells in THA-col compared to THA (Figure 10.7.1A, 3 independent scorers, 3 samples/group, 2slides/group). The col group had the highest score, likely due to presence of cell-binding domains and lower modulus.

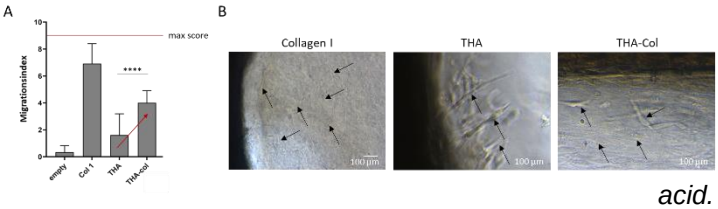


Figure 10.7.1: Chondrocyte migration into acellular hydrogels on day 21. A) Scoring of chondrocyte migration into acellular hydrogels after 21 days of culture. Col1: collagen I isolated from rat tails, THA: tyramine modified hyaluronic acid. B) Brightfield images illustrating cells invading the biomaterial implanted in the cartilage ring (indicated by black arrows). Scale bar 100 µm.

The results demonstrated that the biomaterial composition has an influence of cell invasion into acellular hydrogels. More specifically, the addition of col to THA increases the migration index more than two-fold compared to THA.

Pres:
Schwab A, Alini M, Eglin D, D'Este M. 3D printing of collagen fibrils with controlled orientation within a hyaluronan matrix as biomimetic cartilage implant, TERMIS-EU (cancelled), eCM Meeting Abstracts 2020, Collection 1, page 12.
Schwab A, Alini M, Eglin D, D'Este M. 3D Drucken von Kollagenfibrillen mit Kontrolle über deren Ori-entierung in einer Hyaluronsäure Matrix als biomimetisches Knorpelimplantat, DKOU 2020 oral, Z Orthop Unfall 2020;158 Suppl 1, doi: 10.1055/s-0040-1717553.
Schwab A, Staubli F, Alini M, Eglin D, D'Este M. Einfluss der anisotropen Faserausrichtung und des Kollagen Typs und auf das chondrogene Differenzierungsverhalten, DKOU 2020 poster, Z Orthop Un-fall 2020;158 Suppl 1, doi: 10.1055/s-0040-1717238.
Schwab A, Alini M, Eglin D, D'Este M. Tissue mimetic hyaluronan bioink containing organized collagen fibers to control cell behavior, WBC 2020, online conference.
News article on the AO webpage. Examining the state of the art in bioinks for future Biofabrication re-generative therapies, 04/09/2020.
https://www.aofoundation.org/who-we-are/about-ao/news/2020/2020_09_04_ari_printability

Dissertation:
Flurina Staubli**,** Printability and chondrogenic differentiation of human mesenchymal stromal cell spheroids embedded in collagen-hyaluronan composite inks; Msc ETH Biomedical Engineering, Supervisors: Dr Andrea Schwab, Prof Matteo D'Este; Tutor: Prof Marcy Zenobi-Wong.
Pub:
Schwab A, Levato R, D'Este M, Piluso S, Eglin D, Malda J. Printability and Shape Fidelity of Bioinks in 3D Bioprinting. *Chemical Reviews* **2020**, DOI:10.1021/acs.chemrev.0c00084 10.1021/acs.chemrev.0c00084.
Schwab A, Helary C, Richards RG, Alini M, Eglin D, D'Este M. Tissue mimetic hyaluronan bioink containing collagen fibers with controlled orientation modulating cell migration and alignment. *Mater Today Bio* **2020,** DOI: 10.1016/j.mtbio.2020.100058.

Partners
- Levato R (PhD), Malda J (Prof), The University Medical Center Utrecht, Netherlands
- Bastiaansen-Jenniskens YM (PhD), The University Medical Center, Rotterdam, Netherlands
- Narcisi R (PhD), van Osch G (Prof), The University Medical Center Rotterdam, Netherlands
- Ho K (MD, PhD) & Qin L (Prof, MD), Chinese University of Hong Kong, Hong Kong
- Burdick J (PhD) & Mauck R (Prof), University of Pennsylvania, USA

10.8 AO Development Incubator

Biphasic Plating – a new stabilization concept to improve fracture healing (Biphasic Plate) (Ongoing) (L Hofmann-Fliri, M Windolf)

Background: Severe trauma to the extremities is a leading cause of disability during the wage-earning period with loss of working capacity representing more than 60% of the total cost related to fractures, while the direct cost of medical treatment is less than 20%. Optimal outcomes require not only solid union but also early and complete recovery of limb function. The current generation of fracture fixation plates focuses on minimizing the impact of surgery and preserving the biological healing potential. However, their design poorly controls a second critical component – the mechanical environment of the fracture. Furthermore, these plates are prone to failure, which limits function and delays return to work. The biphasic plating concept proposed by ARI in collaboration with QUT (Brisbane, Australia) was proven by mechanical testing, computer simulations and preclinical experiments.
Goal: To develop and obtain CE Mark of a biphasic anatomical plate for distal femur fractures as a pilot implant and to collect clinical evidence demonstrating the concept feasibility.
Results: After production of the zero series, validation and verification (V&V) testing with a final design and fixed supply chain took place. Performance testing confirmed the superior strength of the Biphasic Plate Distal Femur (more than double fatigue resistance) compared to a standard implant as well as its defined flexibility for fracture healing. Following the V&V activities, the technical documentation was finalized according to MDD and submitted to the Notified Body in Q4 of 2020, expecting a decision in Spring 2021. The Biphasic Plate Distal Femur may hence be available to selected clinics in Europe by Summer 2021.

Figure 10.8.1: Prof Dankward Höntzsch during a usability lab implanting the Biphasic Plate Distal Femur.

Pub:
Hofmann-Fliri L, Epari DR, Schwyn R, Zeiter S, Windolf M. Biphasic Plating - *in vivo* study of a novel fixation concept to enhance mechanobiological fracture healing. Injury. 2020;epub.

Epari DR, Gurung R, Hofmann-Fliri L, Schwyn R, Schuetz M, Windolf M. Biphasic plating improves the mechanical performance of locked plating for distal femur fractures. J Biomech. 2020;epub.

Partners:
- Epari D (Prof), Queensland University of Technology, Brisbane, Australia
- Schütz M (Prof), Jamieson Trauma Institute, Brisbane, Australia

AO Fracture Monitor (SmartPlate) (Ongoing) (M Ernst, M Windolf)

Background: Information on healing progression and load-bearing characteristics in fracture patients is only barely tapped due to the inaccessibility of a confined biological region and the limited value of radiographic methods. A novel approach to continuously measure implant load and patient activity has been recently developed in ARI. The system comprises an implantable data logger, which autonomously collects relevant parameters to support surgical decision-making during fracture healing. Wireless synchronization of the assessed implant load data via the patient's mobile phone allows for remote monitoring by the treating physician. Proof of concept is obtained from preclinical experiments and from first clinical data collection with prototype devices mounted on external fixators.
Goal: The AO Fracture Monitor shall be further developed into a commercially applicable system for long-bone bridge plating. Implantable device and accompanying software shall be developed and tested according to the regulatory requirements and undergo clinical evaluation thereafter.
Results: To date, the most recent version of the AO Fracture Monitor has been applied in sixteen sheep with a tibial osteotomy model stabilized with either 4.5 or 5.5 LCPs. All implantable devices delivered uninterrupted implant load data throughout their lifetime enabling tracking of the progressive bony consolidation. Clinical data collection using a modified fracture monitor device with external fixation has been completed. The results endorse the clinical feasibility of the measurement concept.
Along with final touches to the implant design, manufacturing processes have been developed and are currently being validated. Software components of the system have been further refined in terms of functionality, usability, robustness, and data protection. A registrational, multi-center clinical investigation is currently in preparation.

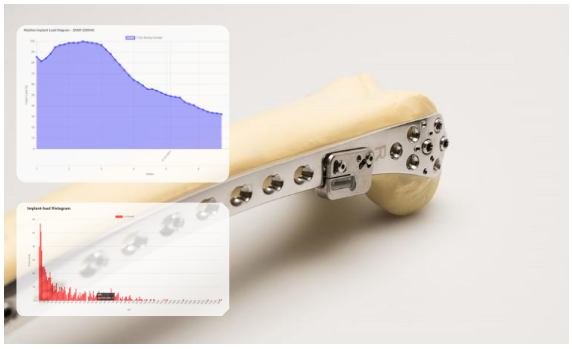


Figure 10.8.2: Implantable data logger attached to an LCP DF plate and exemplary data curves as provided in the cloud environment.

Pres:
Ernst M, Beyond wearables sensors: the AO Fracture Monitor as an implantable device to monitor bone healing, ORS 2020 Annual Meeting, Phoenix, USA (invited oral).
Richards RG, Smart surgery – the AO Fracture Monitor, 46th Annual Meeting of the Japanese Society for Fracture Repair (JSFR2020), online (invited oral).

Pub:
Ernst M, Baumgartner H, Döbele S, Höntzsch D, Pohlemann T, Windolf M. Clinical feasibility of fracture healing assessment through continuous monitoring of implant load. J Biomech. 2020;epub.
Ernst M, Richards RG, Windolf M. Smart implants in fracture care – only buzzword or real opportunity? Injury. 2020;epub.
Windolf M, Ernst M, Schwyn R, Arens D, Zeiter S. The relation between fracture activity and bone healing with special reference to the early healing phase – a preclinical study. Injury. 2020;52(1):71.

Partners:
- Braun B (MD), BG Unfallklinik Tübingen, Germany
- Pohlemann T (Prof), UK Homburg, Germany

3D-SIM

Background: 3D-SIM, 3D sound induced morphogenesis.
Goal: the main goals are: 1) development of an acoustic patterning device (bioprinter), 2) optimization of hydrogels for patterning and definition of biomaterials and labware portfolio for mimix; 3) first *in vivo* anastomosis assessment of spatially organized vascular network.
Results: Mimix launched the first acoustic bioprinter, named CymatiX, on the market on Dec 15th 2020 (Figure 10.8.3). Optimization of patterning parameters for the fabrication of spatially organized vasculature is ongoing Figure 10.8.4.

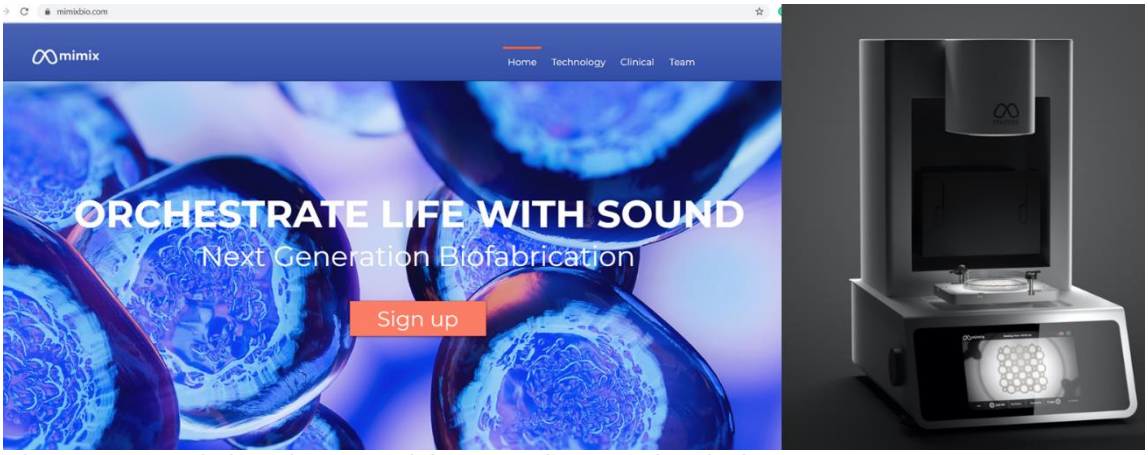


Figure 10.8.3: Mimix webpage and the acoustic patterning device.

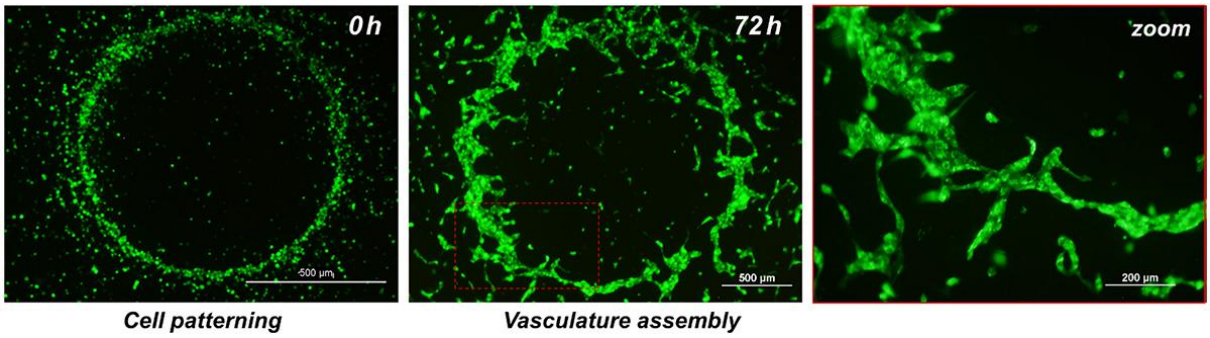


Figure 10.8.4. Human Umbilical Vein Endothelial Cells (HUVEC) patterned within fibrin gel in a circle shape in order to assemble a proto-vessel structure after 72h.

Partners:
- AO Research Institute Davos
- mimiX Biotherapeutics
- Ehrbar M (Prof), UH Zurich

10.9 AO Strategy Fund

Digitally enhanced hands-on surgical training (DEHST) (Ongoing) (J Buschbaum, M Windolf)

Background: Outcomes in orthopedic trauma surgery are highly determined by the skills and training level of the operating surgeon. Hands-on and tactile exercises are an essential pillar of a comprehensive training concept. Classical hands-on training is stationary, limited to basic skill training and lacks data collection. Current digital technologies offer strong opportunities to augment known predominantly mechanical training models with enhanced training scope, user experience and comprehensive training data assessment. They allow to decentralize the training – if desired – from course events to home-based training at any time.
Goal: To develop a skill station product line consisting of low cost, transportable and digitally augmented modules for hands-on surgical training targeting the most relevant operational skills in trauma and orthopedics.
Results: A prototype station for practicing the technically demanding task of free-hand distal interlocking was developed. Essentially, the station consists of a simplified, miniaturized model of a C-arm, simulating intraoperative imaging by generating artificial X-ray images, and a proprietary optical tracking system utilizing a conventional video camera to monitor the training. The training scope includes the key steps of perfect circle alignment, drill tip positioning and drilling. Training success is assessed by comprehensive performance metrics, fed back to the user and collected for holistic skills training improvements. Opposed to classical hands-on training solutions, the system measures training outcome and progress, which is fundamental to future skills certification processes, hence, offering potential for integration into other AO skills training and education programs.

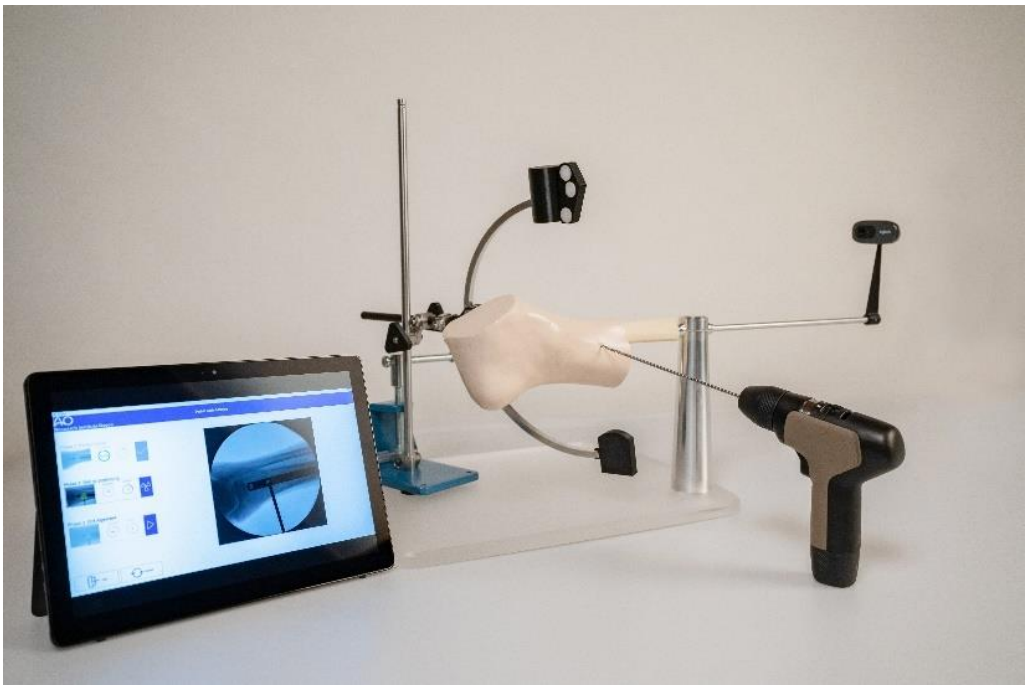


Figure 10.9.1: First developed prototype station for training of free-hand distal interlocking.

Partners:

- Höntzsch D (Prof), Tübingen, Germany
- SYNBONE AG, Zizers, Switzerland

OSapp: Virtual osteosynthesis tool for surgical education (OSAppSF) (Ongoing)
(P Varga, D Mischler, M Knecht, B Gueorguiev, M Windolf)

Background: Osteosynthesis failures are often caused by incorrect surgical techniques. Although the principles of fracture treatment are well taught, less experienced surgeons may lack a sufficient understanding of the underlying biomechanical concepts and therefore fail to identify the correct fixation approach. Improving the mechanical sense and awareness could help surgeons to correctly interpret the fracture situation, develop an appropriate fixation strategy and avoid pitfalls. Therefore, not only the knowledge of the guidelines, but the appropriate understanding of the underlying biomechanical rules is important towards reducing the rate of fixation failures and healing complications.
Goal: Development of a virtual and interactive osteosynthesis learning platform (OSapp) to illustrate and educate the biomechanical principles of fracture fixation and bone healing and foster the understanding of the underlying concepts.
Results: The online version of OSapp has been developed and released at the AO Davos Courses 2020. The content is freely available to registered users at https://osapp.ch/. The Free Configurator module enables unsupervised virtual osteosynthesis exercises via an intuitive and user-friendly interface using simplified and relevant 3D models. The user can configure the fracture type, implant configuration and loading mode, and instantly see the biomechanical outcomes from computer simulations. Understanding and learning are fostered by comparing different configurations. The *Principles* module includes guided learning content to interactively demonstrate fracture fixation principles, i.e. the effect of plate working length. The *Cases* module present virtual case discussions augmented with 3D models to explain the biomechanical rationale behind implant failures or healing complications. Alternative fixation solutions can be configured virtually to demonstrate how the failures could have been avoided and bone healing optimized. The content of OSapp is continuously extended under the guidance of the Medical Advisory Board.

Figure 10.9.2: User interface of the Free Configurator module in OSapp (https://osapp.ch/).

Pub:
Lambert S, Mischler D, Windolf M, Regazzoni P, Dell'Oca AF, Gueorguiev B, Varga P. From creative thinking to scientific principles in clinical practice. Injury. 2021;52(1):32.

Partners:
- Lambert S (MD), University College London Hospital, UK
- Babst R (Prof), Kantonsspital Luzern, Switzerland
- Gebhard F (Prof), Universitätsklinikum Ulm, Germany
- Jaeger M (MD), University Medical Center Freiburg, Freiburg, Germany
- Schütz M (Prof), Jamieson Trauma Institute, Brisbane, Australia

10.10 Extramural Projects

Integrated angular stable locking in the novel Tibia Nail Advanced in combination with low-profile retaining locking screws improves fixation stability in a distal tibia fracture model (Locknail Inlay) (I Zderic, B Gueorguiev)

Background: Unstable distal tibia fractures are challenging injuries requiring surgical treatment. Intramedullary nails are one of the implant options; however, insufficient fixation of the distal fragment may lead to postoperative loss of reduction, delayed healing, malunion or nonunion. Recently, a novel design for angular stable locking has been developed that maintains the basic principle of intramedullary nailing, i.e. relative stability, but introduces improvements expected to reduce nail toggling, screw migration and secondary loss of reduction, without the requirement for additional intraoperative procedures. Core design features are polyether ether ketone (PEEK) inlays integrated in the proximal and distal canal portions of the nail for angular stable screw locking, and low-profile retaining locking screws with enhanced purchase in the near cortex.
Goal: To compare the biomechanical competence of the novel angular stable Tibia Nail Advanced (TNA) concept versus the conventional non-angular stable Expert Tibia Nail (ETN) fixation in a human cadaveric model of an unstable distal tibia fracture under dynamic loading.
Results: Ten pairs of fresh-frozen human cadaveric tibiae with a simulated AO/OTA 42-A3.1 fracture were assigned to two groups for reamed intramedullary nailing using either a non-angular stable ETN with 3 distal screws or the novel TNA with 2 distal angular stable low-profile retaining locking screws. Testing conditions included quasi-static and progressively increasing combined cyclic axial and torsional loading in internal rotation until failure of the bone-implant construct, with monitoring by motion tracking. From a biomechanical perspective, the novel angular stable intramedullary nail concept has the potential of achieving a higher initial axial and torsional stability and maintaining it with a better resistance towards loss of reduction under dynamic loading, while reducing the number of distal locking screws, compared to conventional locking in intramedullary nailed unstable distal tibia fractures.

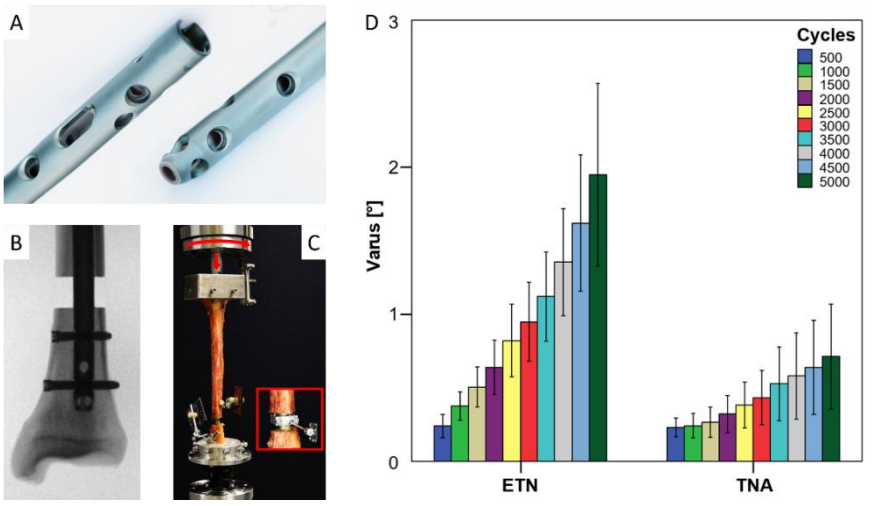


Figure 10.10.1: A) Photographs of the proximal (left) and distal (right) Tibia Nail Advanced (TNA) portions with integrated polyether ether ketone (PEEK) inlays for angular stable screw locking; B) Anteroposterior X-ray of the distal tibia of a specimen with an AO/OTA 42-A3.1 fracture model and a Tibia Nail Advanced (TNA), locked with two angular stable low-profile retaining screws.; C) Setup with a specimen mounted for biomechanical testing; D) Diagram presenting varus interfragmentary movements of the specimens implanted with Expert Tibia Nail (ETN) and Tibia Nail Advanced (TNA) between 500 and 5000 test cycles, demonstrating less varus for TNA compared to ETN, with progressively increasing difference over cycles.

Pres:
Gueorguiev B, Zderic I, Blauth M, Weber A, Koch R, Dauwe J, Schader J, Stoffel K, Finkemeier C, Hessmann M. Angular stable locking in a novel intramedullary nail improves construct stability in a distal tibia fracture model. 2020 EORS (oral).

Partners:
- Hessmann M (Prof), Academic Teaching Hospital Fulda, Fulda, Germany
- Finkemeier C (MD), Orthopaedic Trauma Surgeon of Northern California, Roseville, CA, USA
- Blauth M (Prof), DePuy Synthes, Zuchwil, Switzerland
- Stoffel K (Prof), University Hospital Basel, Basel, Switzerland

Modeling of material injection processes into porous structures applied to vertebroplasty (CemFlow) (Ongoing) (D Gehweiler, E Zweifel, B Gueorguiev)

Background: Vertebroplasty has become an important technique for stabilization of osteoporotic vertebral fractures and other weakening lesions such as angioma or metastatic tumors. However, this procedure presents a significant risk through cement leakage that can result in serious complications such as pulmonary embolism or compressions of nerve roots or the spinal cord. Simulations of the bone cement injection processes could predict injection rates, injection pressures, bone cement distribution within the vertebra and the probability of cement leakage, thus providing a valuable risk assessment tool. However, risk assessment can only be performed if realistic simulations of the entire vertebra are performed.
Goal: To collect experimental data by means of quasi-continuous CT scanning and to model material injection processes applied to vertebroplasty describing bone cement flow behavior and distribution, biomechanical behavior at the interface between bone cement and trabecular structure, and bone cement curing.
Results: A custom-made bone cement injector was used for first experiments. Aluminum foam samples with structure simulating trabecular bone were injected with standard polymethylmethacrylate (PMMA) bone cement. The force applied to the plunger of the syringe during CT scanning was recorded at 10 Hz. An animation of the 3D cement expansion was created from the recorded CT image data and will serve for parametrization and validation of the numerical models at the University of Stuttgart. In addition, a high resolution peripheral quantitative computed tomography (HR-pQCT) scan was performed before and after cement injection.

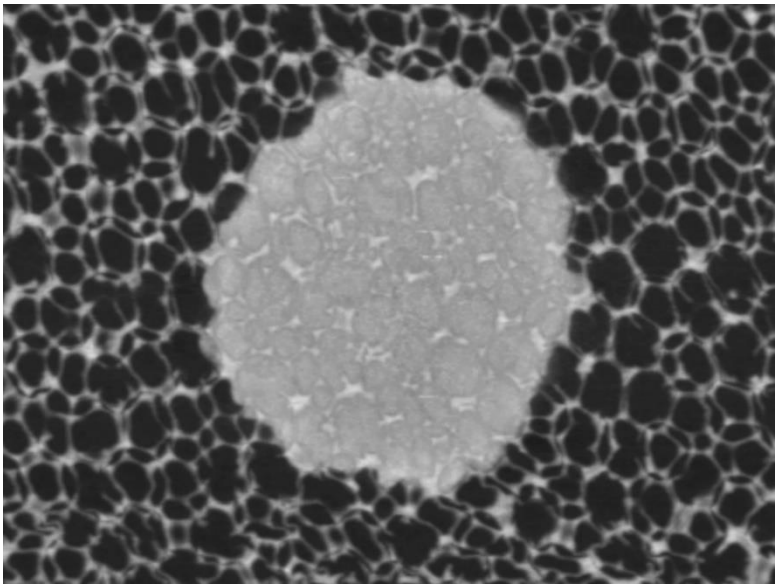


Figure 10.10.2: HR-pQCT image (resolution 0.8 mm) with a projected slice thickness of 1.5 mm visualizing the cement cloud after injection within the aluminum foam.

Pres:
Trivedi Z, Bleier C, Gehweiler D, Gueorguiev-Rüegg B, Ricken T, Wagner A, Röhrle O. Simulating vertebroplasty: a biomechanical challenge. 2020 GAMM (oral).

Partners:
- Röhrle O (Prof), University of Stuttgart, Germany
- Wagner A (Prof), University of Stuttgart, Germany
- Trivedi Z, University of Stuttgart, Germany

The tissue-renin-angiotensin system as novel therapeutic target in the treatment of intervertebral disc degeneration (ProtectDisc) (Started) (Z Li, S Grad)

Background: Recently, our group identified the expression of a tissue Renin-Angiotensin System (tRAS) in human intervertebral discs (IVDs). This system contains two regulatory arms: a pathological proinflammatory arm containing the Angiotensin Converting Enzyme (ACE)/Angiotensin II (AngII)/Angiotensin II receptor type 1 (AGTR1) axis and a protective anti-inflammatory arm containing the Angiotensin II receptor type 2 (AGTR2)/Ang1-7/MasReceptor axis (Figure 10.10.3A). The expression of the pathologic arm was correlated with the extent of inflammation and tissue degeneration in our previous study, indicating an involvement of this system in intervertebral disc degeneration (IDD).
Goal: This project aims to investigate the functional characteristics of the regulatory arms and the therapeutic relevance of tRAS modulation for IDD. The impact of pathologic tRAS arm inhibition and protective tRAS arm stimulation for inflammation management and treatment of IDD will be investigated.
Results: No cytotoxic effects of angiotensin II were observed in the cell viability analysis for the examined dose ranges and durations (Figure 10.10.3B). AGTR1, AGTR2, and MAS receptors were identified in human NP cells (Figure 10.10.3C). The addition of angiotensin II, regardless of the concentration or combination with TNF-α, did not show any significant effect on gene expression of inflammatory (TNF-α, IL-6, IL-8, TLR4), anti-inflammatory (IL-10), regenerative/ degenerative (ACAN, COL1, MMP1, MMP3) or tRAS markers (AGTR1, ACE, AGTR2, MAS1) in human NP cells. However, there was a significant increase of NO secreted by the cells in the angiotensin II exposed groups compared to the control ($p<0.05$) or TNF-α ($p<0.01$) only group (Figure 10.10.3E). The gene expression ratios of pro-inflammatory/anti-inflammatory cytokines IL-6/-10, IL-8/IL-10, and the TNF-α/IL-10 were positively correlated with the AGTR1/AGTR2 and AGTR1/MAS1 ratios, respectively (Figure 10.10.3D).

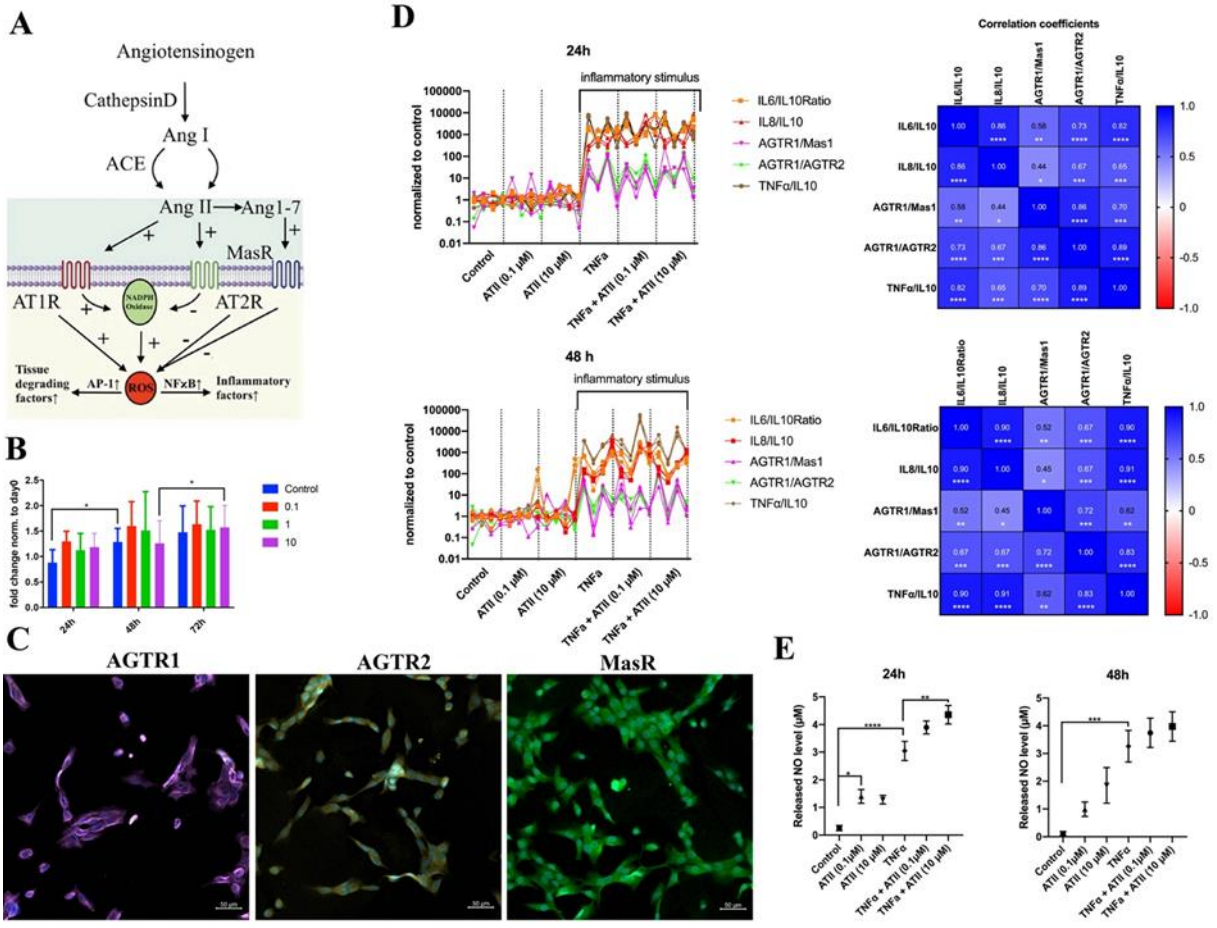


*Figure 10.10.3: (A) Illustration of potential tissue renin-angiotensin system pathways in human nucleus pulposus (NP) cells. AngI: angiotensin I; ACE: angiotensin-converting enzyme (B) Cell viability was not affected by angiotensin II (ATII) (0.1-10 µM) after 72 h of exposure. N=6, mean+sem, *p<0.05. (C) Immunofluorescent staining images of angiotensin II type 1 (AGTR1), type 2 (AGTR2), and MAS receptor (MasR) in human NP cells. (D) Gene expression ratios of AGTR1/AGTR2 and AGTR1/MAS1 were positively correlated with the pro-inflammatory/anti-inflammatory marker ratios IL-6/IL-10, IL-8/IL-10, and TNF-α/IL-10. Spearman correlation coefficients are shown in the matrix. *p<0.05; **p<0.01; ***p<0.001;****p<0.0001. (E) TNFα + ATII exposure for 24 hours showed increased nitric oxide (NO) release by the cells compared to the TNF-α only group. N=12, mean±sem, *p<0.05; **p<0.01; ***p<0.001;****p<0.0001.*

Fund: EUR 50,000 (EUR 30,000 (DWG) / EUR 20,000 (DAH)); 2020-2021

Pres:
Pfannkuche J, Li Z, Grad S, Alini M, Häckel S, Südkamp N, Schmal H, Kubosch D, Lang G. Anti-inflammatory effects of Losartan in human nucleus pulposus cells. EUROSpine, Oct 6-9, 2020 (e-presentation).
Li Z, Pfannkuche J, Kubosch D, Südkamp N, Alini M, Grad S, Lang G. Angiotensin II receptor antagonist inhibits TNFα induced degeneration process in human nucleus pulposus cells. ORS, Feb 8-11, 2020 (poster).

Pub:
Saravi B, Lang G, Ülkümen S, Burchard T, Weihrauch V, Patzelt S, Boeker M, Li Z, Woelber JP. The tissue renin-angiotensin system (tRAS) and the impact of its inhibition on inflammation and bone loss in the periodontal tissue. Eur Cell Mater. 2020; 40:203-226.
Li Z, Wystrach L, Bernstein A, Grad S, Alini M, Richards RG, Kubosch D, Südkamp N, Izadpanah K, Kubosch J, Lang G. The tissue-renin-angiotensin-system of the human intervertebral disc. Eur Cell Mater. 2020; 40:115-132.
Pfannkuche JJ, Guo W, Cui S, Ma J, Lang G, Peroglio M, Richards RG, Alini M, Grad S, Li Z. Intervertebral disc organ culture for the investigation of disc pathology and regeneration - benefits, limitations, and future directions of bioreactors. Connect Tissue Res. 2020; 61:304-321.
Li Z, Gehlen Y, Heizmann F, Grad S, Alini M, Richards RG, Kubosch D, Südkamp N, Izadpanah K, Kubosch EJ, Lang G. Preclinical ex-vivo testing of anti-inflammatory drugs in a bovine intervertebral degenerative disc model. Frontiers in Bioengineering and Biotechnology. 2020; 8:583.

Partners:
- Lang G (MD), Department of Orthopedics and Trauma Surgery, University Hospital Freiburg, Freiburg, Germany

Induced pluripotent stem cell-based therapy for spinal regeneration (iPSpine) (Ongoing) (S Grad, A Vernengo)

Background: This multicentre project aims to develop and demonstrate the Proof-of-Concept for a novel induced pluripotent stem cell (iPSC)-based therapeutic strategy as a regenerative therapy. iPSpine is targeting a societal challenge affecting millions of people, i.e. low back pain caused by intervertebral disc degeneration. The *iPSpine* team will: 1) differentiate iPSCs towards notochordal-like cells which are specialised tissue specific progenitor cells with a critical role in rejuvenating the intervertebral disc; 2) develop smart biomaterials as a conductive microenvironment to prime iPSCs towards notochordal-like cells and instruct intervertebral disc regeneration, and 3) demonstrate the safety and efficacy of the *iPSpine* advanced therapy in clinically relevant pre-clinical models.
Goal: The aim of the ARI investigators is to create a suitable organ culture model and test the iPSC-based therapy in this preclinical ex-vivo setting.

Results: A bovine organ culture model was generated using papain injection and the influence of dynamic loading was analysed. Injection of papain created a cavity, resulted in extracellular matrix disorganization, and reduced tissue glycosaminoglycan content in the loaded and free swelling samples. Furthermore, daily dynamic loading induced a significant reduction in disc height and volume. Relative gene expression was downregulated for anabolic markers, while catabolic markers were upregulated. The results suggest that the papain-induced degeneration model is suitable for implantation of regenerative therapeutic biomaterials and cells.

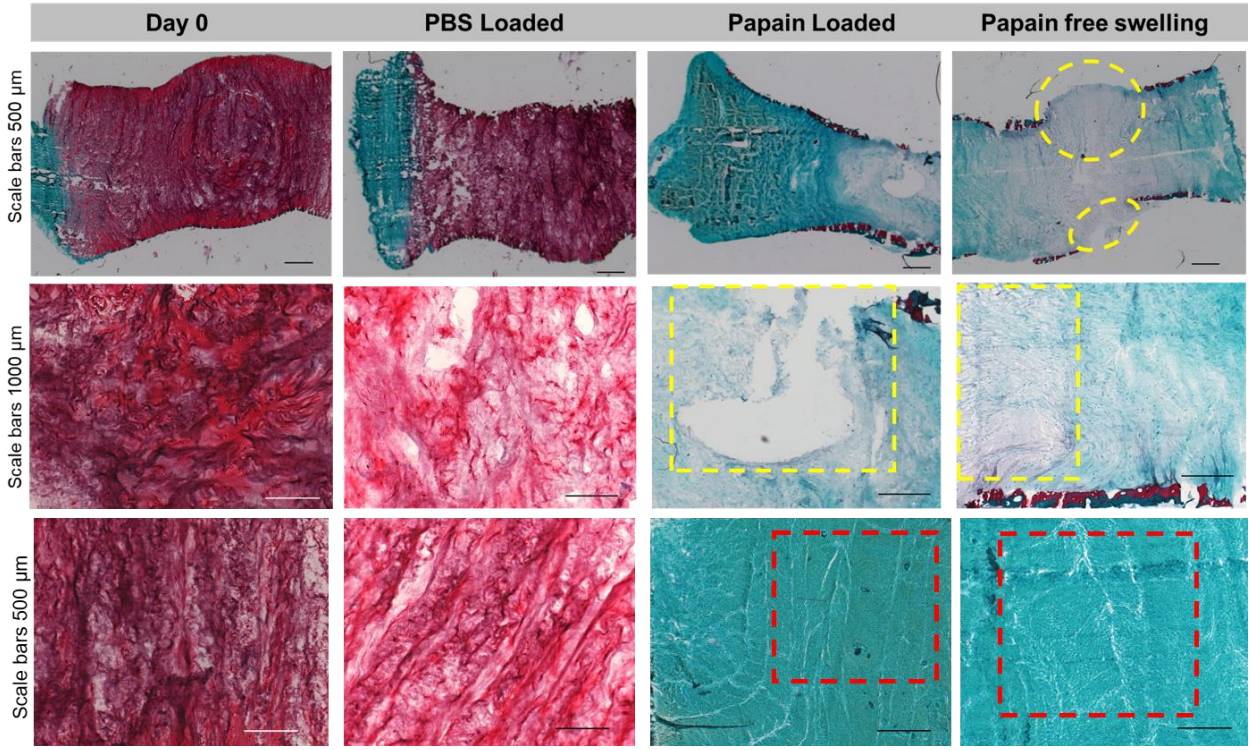


Figure 10.10.4: Safranin O/Fast Green Staining at Day 7 of Papain Digestion for PBS loaded, papain loaded and papain free swelling specimen. Day 0 sample is showed as control. Regions of matrix depletion and fissures are indicated.

Fund: EU H2020-SC1-BHC-2018-2020 RIA- Grant; ARI Funding EUR 491,250; Period: 2019-2023

Pres:
Kluser N, Vernengo A, Jansen JU, Neidlinger-Wilke C, Wilke HJ, Li Z, Grad S. Papain-induced *ex vivo* model for intervertebral disc degeneration and the influence of daily physiological loading. 2020 Graubünden Forscht virtual (oral).

Partners:
- Tryfonidou M (Prof), University of Utrecht, Netherlands
- Creemers L (PhD), University Medical Centre Utrecht, Netherlands
- Ito K (Prof), Technical University of Eindhoven, Netherlands
- Guicheux J (Prof), University of Nantes, France
- Pandit A (Prof), National University of Galway, Ireland
- Wilke H-J (Prof), University of Ulm, Germany
- Gantenbein B (Prof), University of Bern, Switzerland
- Jorgensen C (Prof), Institute National de la Sante, France
- Templin M, Naturwissenschaftliches und Medizinisches Institut, Germany
- Le Maitre C (Prof), Sheffield Hallam University, UK
- Vadala G, University Campus Biomedico, Rom, Italy
- De Boer M, Ntrans Technologies, Netherlands

- Noel D, University of Montpellier, France
- Isasi R, University of Miami, US
- Kienle A, Spineserv Gmbh, Germany
- Chan D, The University of Hong Kong, Hong Kong
- Buljovcic Z, Pharmalex Gmbh, Germany
- Lether I, National Reumafonds, Netherlands

In-JOINT APPlication of non-viral mRNA therapy for OsteoArthritis (Joint-Approach) (Ongoing) (S Grad, V Basoli, M Alini)

Background: Osteoarthritis (OA) is characterized by chronic joint pain and functional impairment and imposes a huge burden on the individual patient and health care systems. Current treatments relieve symptoms, but do not counteract disease progression. Intra-articular (in-joint) gene therapy, such as mRNA therapy offers a promising highly innovative solution for the treatment of OA.
Goal: This project proposes a novel approach, using polymer nanoparticle-based delivery of stabilized mRNA candidates. The patented nanotechnology of 20MED is combined with the proprietary stabilized non-immunogenic mRNA technology of ETHRIS, to deliver a non-viral mRNA-based 'transcript therapy' for injection into the joint. The preclinical efficacy will be tested in ex-vivo joint bioreactors and rat disease models by ARI and Paracelsus Medical University.
Results: Suitable cartilage inflammation models were developed, and therapeutic mRNA transfection was studied and optimized. First, an *in vitro* inflammation model for chondrocytes (2D) was evaluated, and subsequently, the development of the *ex vivo* osteochondral explant organ culture model was started. The efficacy of optimized therapeutic mRNA formulations was demonstrated in 2D with a candidate molecule approach (IL1Ra mRNA), and this approach was then applied to the osteochondral explant model.

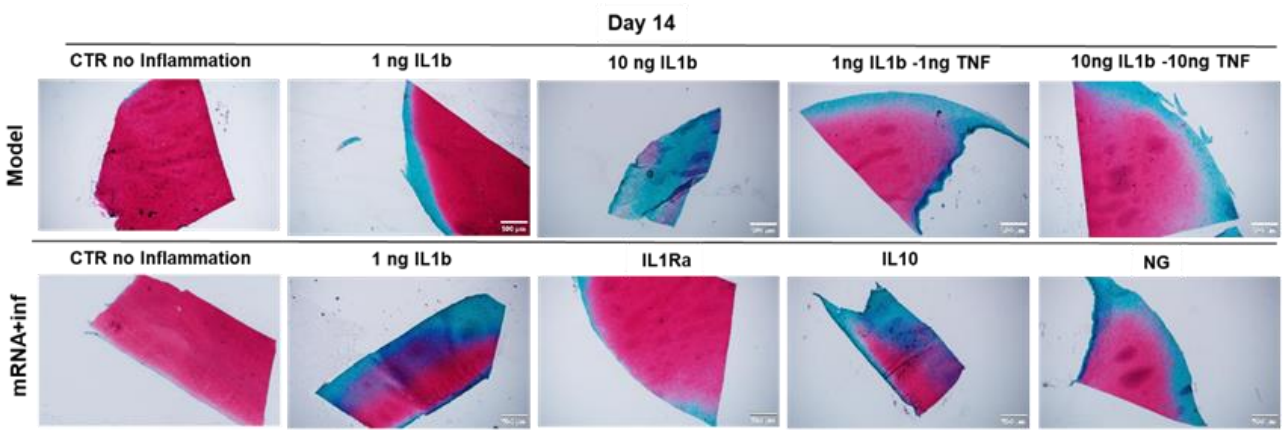


Figure10.10.5: Safranin O/Fast Green stained sections of the inflammatory cartilage explant model (upper panel) and treatment with nanogel (NG) with or without therapeutic mRNAs (IL1Ra; IL10; lower panel)

Fund: Eurostars; ARI Funding EUR 200,000; Period 2019-2022.

Partners:
- Engbersen J (Prof), 20Med Therapeutics, Netherlands
- Planck C (Prof), Ethris Gmbh, Germany
- Traweger A (Prof), Paracelsus Medical University, Austria

Real Time quality monitoring of Engineered Tissue for regenerative medicine (RT-Monet) (Started) (V Basoli)

Background: Despite the growing demand and interest in biofabrication in the field of tissue engineering and regenerative medicine, there are currently no efficient methods for monitoring construct function and behavior over time.
Goal: In collaboration with CSEM (Landquart) we aim to create an innovative method based on insertable micro-biosensors that can screen in real time and non-invasively cellular behavior during the (bio)fabrication and *in vitro* maturation process. We will develop a device with miniaturized sensors that can measure parameters and send the information to a remote controller. The monitoring will aim at quality control (viability, differentiation), to increase the success rate for therapeutic transplantation. Nowadays the most common analysis techniques include the sampling of medium and measurement of analytes by spectrophotometric assays, HPLC or gene expression analysis. These methods do not allow us to follow the cellular quality in real time and remotely. We aim to create an innovative and non-invasive way to study the behavior of cells with ad hoc insertable specific miniaturized micro-biosensors that allow in situ and real-time quality control of tissue engineering constructs. The monitoring will be done on a cellular level on multiple samples (inside tissue culture plates) over culture time by an easy, cost-effective, analytic, and reproducible method using specific markers based on the desired type of observation.

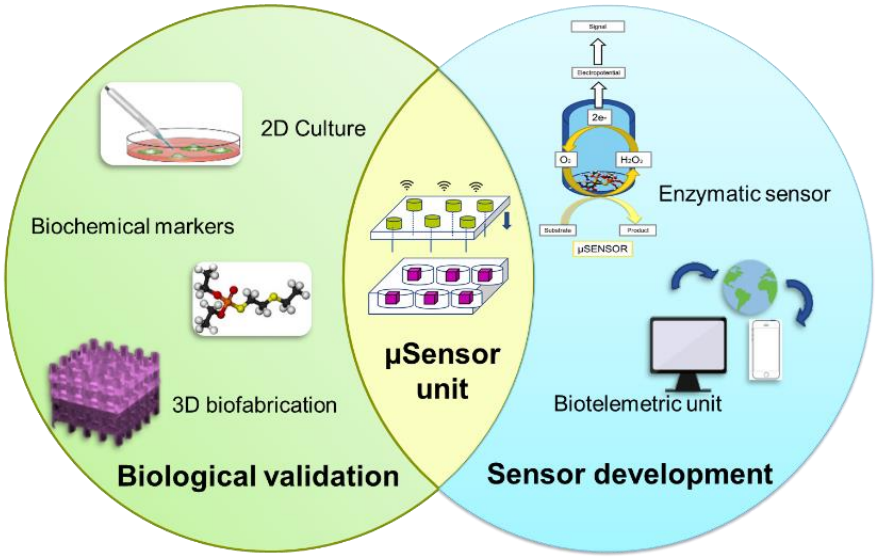


Figure 10.10.6: Outline of the project, whereby the contribution of the ARI team is shown in green, the contribution of CSEM is shown in blue.

Fund: SNF SPARK; CHF 100,000; 2020-2021

Pres:
Zuncheddu D, Generelli S, Kurth F, Serra PA, Rocchitta G, Grad S, Basoli V. Real time quality monitoring of engineered tissue for regenerative medicine. 2020 GR forscht virtual (oral).

Partner:
- Generelli S, CSEM Landquart, Switzerland

Treating Discogenic Pain by Reducing Nerve Sensitization and Ingrowth using the COX-2 Inhibitor Celecoxib – An *in vitro* Study with Inflamed Dorsal Root Ganglion Cells (COX2IVD) (Started) (S Häckel, M Peroglio, S Grad)

Background: Up to 80% of all people will have low back pain (LBP) at least once during their life. At least 20% of these people will move towards chronic disease. The causes of LBP are manifold with one of the main causes a degenerated intervertebral disc (IVD). While healthy IVDs are avascular and poorly innervated, increased neovascularization and innervation have been observed in degenerated discs. Nerve endings in the degenerated disc are activated due to a constant inflammation and are thought to transmit the pain signals to the central nervous system. In pain management of LBP, nonsteroidal anti-inflammatory drugs, and specifically cyclooxygenase-2 blocker like celecoxib, are often prescribed for an oral use and have many side effects.

Goal: The aim of this study is to investigate the interaction between celecoxib in an inflammatory disc environment and its effect on nerve cells. By using an *in vitro* model (with conditioned medium of IVD cells), the effect of celecoxib on outgrowth and sensitization of dorsal root ganglion cells (DRG), which are the main transmitter of pain signals in the IVD, will be investigated. The proof of this specific effect will help to understand celecoxib´s mechanism of action in the inflamed disc environment and could promote its clinical translation.

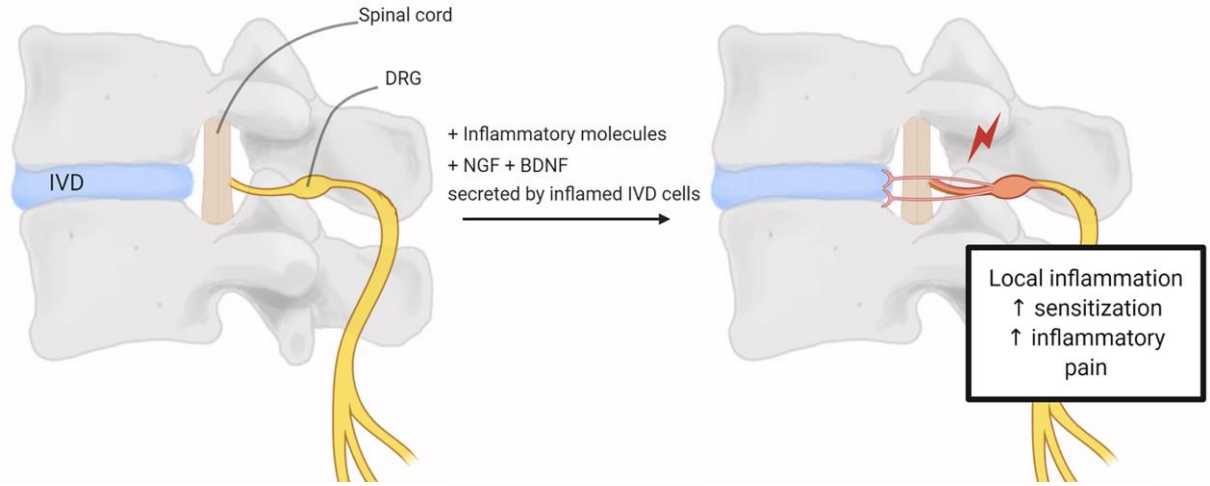


Figure 10.10.7: Sagittal view of the spine. Peripheral nerves entering the spinal cord via the dorsal root ganglion (DRG). Inflammation of the intervertebral disc (IVD) can trigger nerve ingrowth and sensitization. Figure created with Biorender.

Fund: Swiss Orthopaedics; CHF 20,000; 2020-2021

Pres: Häckel S, Häne S, Ma J, Li Z, Pfannkuche J, Peroglio M, Hoppe S, Benneker L, Lang G, Südkamp N, Grad S. Could the COX-2 inhibitor Celecoxib influence discogenic pain? An *in vitro* study with inflamed annulus fibrosus cells. Virtual EFORT Congress (VEC) (Poster).

Dissertation:
Häne SI. Treating Discogenic Pain by Reducing Dorsal Root Ganglion Cell Sensitization using the COX-2 Inhibitor Celecoxib – An *in vitro* Study with Inflammatory Cytokine Treated Annulus Fibrosus Cells.2020 ETH Zurich (S. Grad, U. Suter, Z. Li, J. Ma) – MSc ETH.

- **Partners**:
  Häckel S (MD), Benneker L (Prof), Hoppe S (PD), Inselspital Bern, Bern, Switzerland
- Creemers L (Prof), University Medical Center Utrecht, Utrecht, The Netherlands

Advanced *in vitro* organ degeneration models for musculoskeletal research (Multireact) (Started) (S Grad, M Alini, A Secerovic, A Ristaniemi)

Background: Currently, the translation of research from the lab to the clinic is not reliable due to an oversimplification of the *in vitro* models and limitations of animal testing. The musculoskeletal system incorporates bones, cartilage, skeletal muscles, tendons and ligaments to provide mechanical support and permit movement. Most *in vitro* models provide static or oversimplified dynamic (e.g. only compression) environments over short-term tissue culture periods.
Goal: The overall objective is to develop a multi-axis dynamic *in vitro* system to mimic movement (with a focus on intervertebral discs) for long-term musculoskeletal tissue culture. The disruptive potential of this project can only be reached by the combination of bilateral synergies in tissue regeneration and standardized tissue testing and will result in one advanced *in vitro* system. This interdisciplinary stems from the collaboration of CSEM (6-DOF bioreactor), ETH Zurich (biomechanics) and ARI (*in vitro* and animal models). The first phase concentrates on the validation of a new generation of bioreactor with uniaxial mechanical loading. The second phase will deliver a universal and versatile six degrees-of-freedom bioreactor for long-term musculoskeletal tissue culture. The third phase will generate a new set of degeneration models that are clinically relevant. Our vision for this project is to develop THE new generation of *in vitro* musculoskeletal models for standardized pre-clinical assessment.

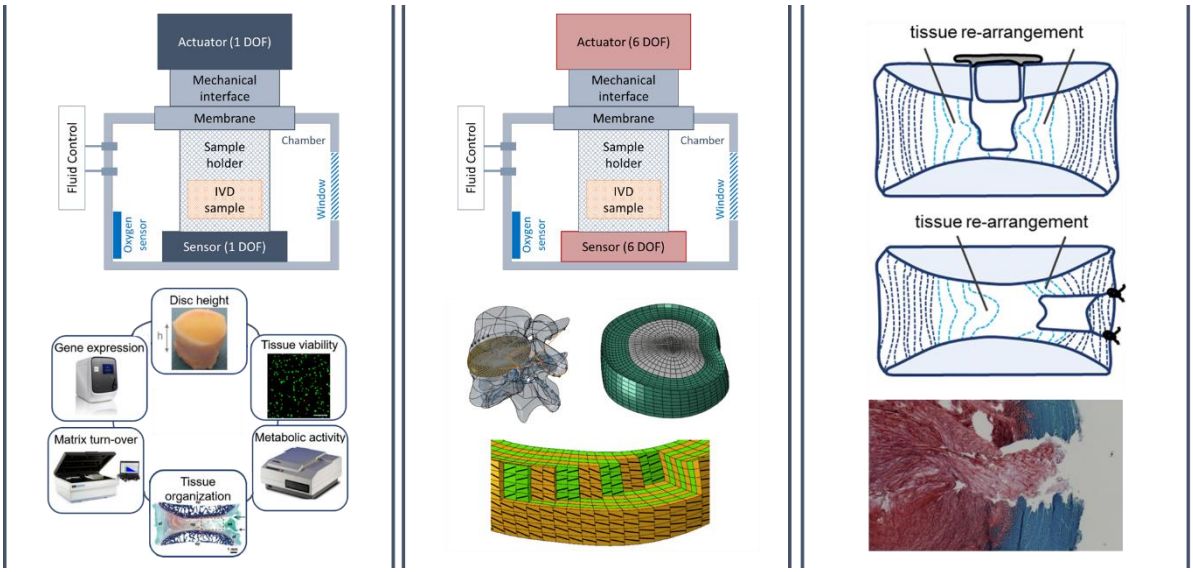


Figure 10.10.8: Three-step approach validating a new compression bioreactor (left), a 6-degree of freedom loading device (center), and new degeneration models (right) of the intervertebral disc.

Fund: SNF Sinergia; CHF 670,410; 2020-2023

Partners:
- Ferguson SJ (Prof), ETH Zurich, Switzerland
- Weder G (Dr), CSEM Neuchatel, Switzerland
- Heub S (Dr), CSEM Neuchatel, Switzerland

Identifying novel therapeutic targets for articular cartilage repair (STEMSEC) (Ongoing) M Stoddart)

Background: Novel therapies for cartilage regeneration have had limited success. Chondrogenic differentiation of mesenchymal stem cells (MSCs) under load is different to that observed during classical static culture conditions. This is highly clinically relevant, considering that patients receive weight-bearing rehabilitation therapy following cartilage repair.

Additionally, as most *in vitro* cartilage repair studies are performed under static conditions, the lack of mechanical stimulation may explain why it has been challenging to reproduce promising *in vitro* results *in vivo*. Marrow stimulation techniques, such as microfracture, are the most commonly used clinical approach for cartilage repair with unpredictable results. Using a unique *in vivo* kinematic join simulating bioreactor, we have previously shown that while complex multiaxial load induces hMSC chondrogenesis, it also induces the expression of a number of soluble molecules not typically found under static culture conditions. This identified novel mechanically induced targets, such as nitric oxide (NO), that are potentially clinically relevant. Within this project we aim to better understand the role of mechanical load on the molecules induced during human MSC chondrogenesis vs standard conditions (static and with transforming growth factor β (TGF-β)). We will identify new potential treatment targets, while investigating the biological function of nitric oxide.
Goal: This project aims to establish the functional modulation of non-cartilage cell types by mechanically stimulated MSC secretome, thus providing valuable further insight into the pathology of joint destruction.
Results: Using a design of experiments (DoE) approach, we have established optimal loading conditions to a) increase TGFβ production and b) increase the mechanical activation of latent TGFβ protein. Interestingly the protocol optimal for expression is not the same as that optimal for activation. This suggests that rehabilitation protocols may need to increase in complexity to improve cellular differentiation.

Fund: Swiss National Funds (nr 31003A_179438 / 1), Funding: CHF 417'720, Period: 08/2018-07/2022

Partner:
- Snedeker Jess G (Prof, PhD), ETH Zurich, Switzerland

3D Printed-Matrix Assisted Chemically Modified RNAs Bone Regenerative Therapy for Trauma and Osteoporotic Patients (cmRNA Bone) (M D'Este, M Stoddart)

Background: Due to lifestyle changes and ageing of our industrialized nations, bone traumatic injuries and osteoporosis induced fragility fractures are an enormous medical and socio-economic challenge. State-of-the-art therapies have failed until now in keeping their promises of reliable bone regenerative solutions. The cmRNAbone project aims to create a novel bone regenerative therapeutic approach based on combination of chemically modified RNAs (cmRNAs)-vectors embedded in a 3D-printed guiding biomaterial ink tailored to patients need. To achieve our goal, Semaphorin 3A (SEMA3a), Vascular endothelial growth factor (VEGF), Platelet-derived growth factor (PDGF-BB) and Bone Morphogenetic Protein 7 (BMP7) cmRNAs targeting neurogenesis, vasculogenesis and osteogenesis will be synthesized. Vectors based on lipids and polysaccharide nanocapsules for cmRNA delivery will also be developed. A functional Hyaluronan-Calcium Phosphate biomaterial ink that 1) can be loaded with cmRNAs-vectors and release them, 2) having intrinsic osteoinductivity and presenting laminin-derived peptides for guiding sensory neurons and endothelial cells ingrowth, and 3) being amenable to an extrusion-based 3D-bioprinting process will be formulated in conjunction with a novel 3Dprinter for fabrication of patient specific regenerative solutions. In the following step, a large effort will focus on deciphering the regenerative mechanisms and optimizing dosage and ratios of cmRNAs, loading of cmRNAs-vectors in the ink, 3D-printing, etc, to demonstrate regenerative capabilities *in vitro* and *in vivo*. Selected candidate formulations will be taken to clinically relevant preclinical proof of concepts. Finally, an overreaching effort on preparing a 1st in human trial will be taken, consisting of partner facilities auditing and clinical experts group support, to ensure that GMP-like production for all regenerative tools, and regulatory and commercial strategies are realized.

Fund: H2020-SC1-BHC-2018-2020. Total Budget €6.26 million, ARI Budget €710k, Period 2020-2023

Partners:

- Stoddart M (Prof), AO Research Institute Davos, Switzerland (Coordinator)
- Banfi A (Prof, PhD), University of Basel, Switzerland
- Plank C (Prof, PhD), ETHRIS GmbH, Germany
- Schepp N, EURICE - European Research and Project Office GmbH, Germany
- Damien D (PhD), CIDETEC, Spain
- de Groot F (PhD), Kuros Biosciences BV, The Netherlands
- Zelphati O, OZ Biosciences SAS, France
- Fernández A (PhD), IDONIAL TECHNOLOGICAL CENTER, Spain
- van Griensven M (Prof, PhD), Maastricht University, The Netherlands
- Amédée J (Prof, PhD), University of Bordeaux, France

Comparison of a high-virulent versus a low-virulent Staphylococcus aureus strain in a murine bone infection model (HiLo) (Started) (S Bärtl, F Moriarty, S Zeiter)

Background*:* *Staphylococcus aureus* is the most common pathogen in fracture-related infections (FRI). It has become evident that irrespective of antibiotic resistance patterns, several virulence factors have a critical impact on the course of infection.
Goal: Determine if virulence observed in human patients translates to histopathological differences in a controlled *in vivo* FRI model.
Results: Based on two patients with significantly different infection presentations, a presumed high-virulent *S. aureus* strain (EDCC5458) and a presumed a low-virulent *S. aureus* isolate (EDCC 5464) were compared in terms of clinical presentation, systemic inflammation, bacteriology, and histopathological evaluation in a mouse FRI model. Time points of interest were set at a short interval (4 days post-surgery) and a longer interval (14-days post-surgery). Bacteriological results of soft tissue samples showed a higher infectious burden in the high-virulent infected animals in the early infection phase (Fig.1). Throughout infection, CFU counts tended to become more similar at day 14. The high-virulent strain exhibited more pronounced systemic dissemination, as elucidated by high rates of infected organs at both time points (Figure 10.10.9). Cytokine analysis in serum samples and histopathological evaluation are currently under way.

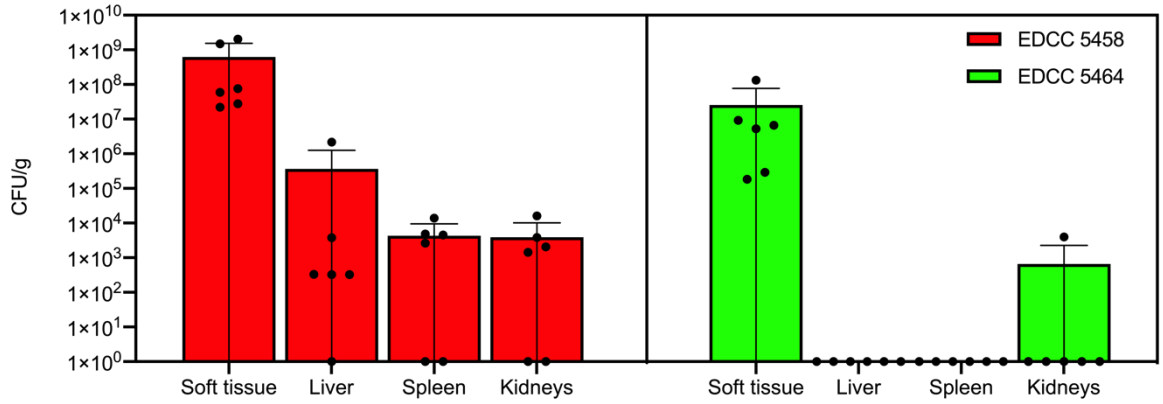


Figure 10.10.9: Quantitative bacteriology from soft tissue and organs at euthanasia of the presumed high-virulence (red) strain and the presumed low-virulence strain (green). These results show day 4-post-surgery. Data are expressed as means and standard deviation of the mean.

Partner:

- Alt V (Prof), University Hospital Regensburg, Germany.

Antibiofilm therapy using Local Application of Bacteriophages (Antibio_LAB) (Ongoing) (F Moriarty, D Eglin, S Zeiter)

Background and goal: The goals of the project are creating a phage collection with isolates targeting biofilms of clinical isolates of *Staphylococcus aureus* (including methicillin resistant *S. aureus,* MRSA) and multi drug resistant (MDR) *Pseudomonas aeruginosa*.
Results: Our achievements to date have included the establishment an international *S. aureus* (MRSA and MSSA) and *P. aeruginosa* (MDR) strain collection. These isolates were collected from patients in Germany, Belgium, and Switzerland. For these isolates, an antibiogram and a genome sequence is available (*P. aeruginosa* collection population structure shown in Figure 10.10.10). Furthermore, a phage collection was established by isolating new phages from sewage and from saliva and nasal swab samples collected from team members of the different research partners (Davos, Switzerland; Leuven, Belgium; Berlin, Germany). The host spectrum of all PA phages towards all PA clinical isolates was determined and 4 phages were selected based on genome analysis and host spectrum for the natural *in vitro* evolution.

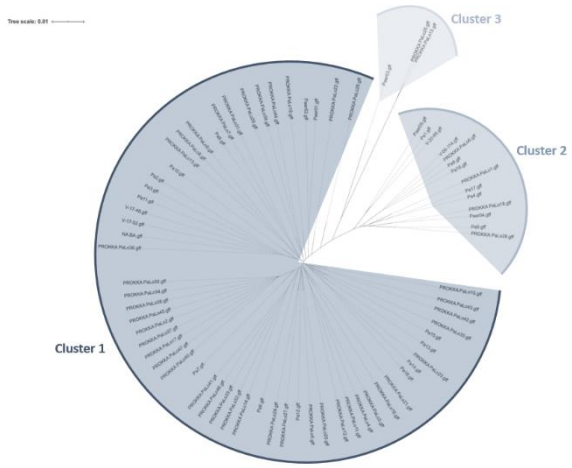


Figure 10.10.10: Population structure of the P. aeruginosa isolates collected within the frame of this consortium project based on an alignment of the core genes.

Pub:
Rotman S et al *Local Bacteriophage Delivery for Treatment and Prevention of Bacterial Infections.* Frontiers in Microbiology 2020.

Partners:
- Rob Lavigne (KU Leuven, Belgium)
- Andrej Trampuz MD (Charite Berlin, Germany)
- Willem-Jan Metsemakers MD (KU Leuven, Belgium)

3D Printed Multi-Scale, Cell Instructive Tissue Engineering Scaffolds for Annulus Fibrosus Tissue Regeneration (MultiscaleAF) (Started) (A Vernengo)

Background: Intervertebral disc (IVD) degeneration is characterized by fissures or ruptures in its peripheral annulus fibrosus (AF). Current clinical approaches to AF closure do not address the long-term need for functional tissue to prevent or postpone further degeneration. The proposed research is aimed at advancing the state of AF tissue engineering. The AF is a complex, multilamellar structure comprised of fibers which possess a gradient in composition, angular orientation, and cell phenotype from the outer towards the inner AF. We have developed a melt-extrusion-based 3D printing method for the fabrication of multi-scale tissue engineering scaffolds with defined surface topographies that guide cellular organization. Polycaprolactone (PCL) was thermally extruded through custom-designed printer nozzles possessing varying circumferential sinusoidal patterns (smooth, 30, 60, or 120 μm peak height). Extrusion through the nozzles resulted in cylindrical struts possessing longitudinally aligned surface grooves.
Goal: The aim of this study is to evaluate mesenchymal stem cell (MSC) lineage commitment to AF phenotypes mediated by longitudinally aligned grooves on PCL surfaces. We hypothesize that: 1) Longitudinal surface grooves can serve as biophysical cues to induce cell alignment and the deposition of ECM along the surface pattern; 2) Increasing peak height of the grooves can be used to tune MSC differentiation towards phenotypes of the inner, middle or outer AF, resulting in ECM deposition mimicking the regional tissue organization and composition.

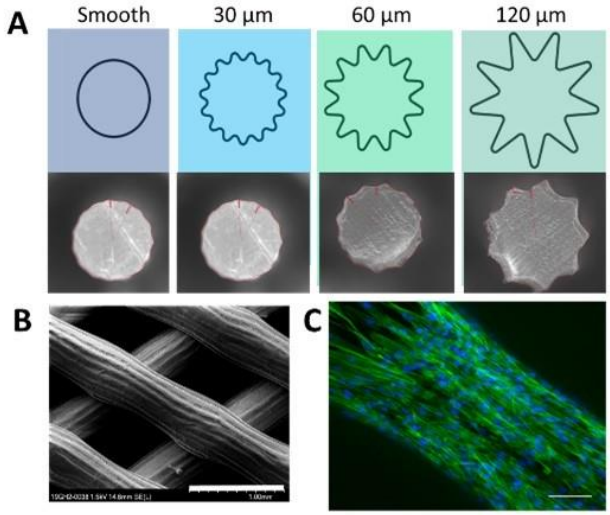


Figure10.10.11: (A) Nozzles possessing circumferential sinusoidal patterns with varying peak heights (30, 60, 120 µm) were used to extrude cylindrical struts with longitudinally aligned surface grooves. (B) Top view of the microscale topographical features used to guide cell alignment. Scale bar 50 µm. (C) Phalloidin/DAPI stained MSCs seeded on the PCL scaffolds printed with the 60 µm nozzles. Scale bar 100 µm.

Fund: ON Foundation; CHF 10,000; 2020-2021.

Pub:
Vernengo AJ, Grad S, Eglin D, Alin M, Li Z. Bioprinting Tissue Analogues with Decellularized Extracellular Matrix Bioink for Regeneration and Tissue Models of Cartilage and Intervertebral Discs. Adv. Funct. Mater. 2020, 1909044. DOI: 10.1002/adfm.201909044.

Dissertation:
Kluser N. 3D Printed Multi-Scale Scaffolds with Topographical Guidance for Annulus Fibrosus Regeneration.2020 ETH Zurich (A Vernengo, S Grad, S Ferguson) – MSc ETH HAST.

Partner:
- Häckel S (Dr), Inselspital Bern

Baltic Biomaterials Centre of Excellence (BBCE) (Ongoing) (M D'Este, N Goudsouzian, M Alini)

Background: According to recent studies, Latvia is the 4th from the bottom in Research excellence performance compared to the other EU countries. Scores of the Research excellence indicators show that currently Latvia is significantly below the EU27 average performance in Science and Technology (S&T) Excellence. The total R&D expenditure in percentage of Gross Domestic Product (GDP) in Latvia, both public and private combined, has been one of the lowest in Europe rating almost 4 times lower than the EU average. In addition, given geopolitical instability, residual funds of public financing will be devoted mostly for the defense issues, whereas R&D funding will not be increased significantly.
Goal: The Baltic Biomaterials Centre of Excellence (BBCE) overall objective is to develop a joint BBCE for advanced biomaterials development based on the long-term strategic cooperation between Riga Technical University, Latvian Institute of Organic Synthesis, Rīga Stradiņš University and LLC Rīga Stradiņš University Institute of Stomatology, on the one part, and the ARI plus Friedrich-Alexander University of Erlangen-Nuremberg, Germany, on the other part.

Results: The activities of the BBCE will provide an opportunity to combine existing expertise and infrastructure to create critical mass and excellence in the development of biomaterials for bone regeneration and solutions for creative biomedical applications. The expected impact of the BBCE project through Teaming Phase 2 in long term will be achieved through fruitful cooperation between the BBCE core partners in Latvia and industry (including SMEs) bringing products into the market, increasing scientific excellence, elevating the impact factor of peer-reviewed publications and Hirsch index in the field of biomaterials. Collecting "critical mass" of high-level scientists and/or technology developers will be achieved ensuring career

development to provide highly qualified staff at BBCE core partners able to work in a multinational and interdisciplinary environment and capable to cope with their future career demands in an efficient and innovative way. This project is receiving 15 M funding from the European Union's Horizon 2020 research and innovation program and the Latvian Government, with 15 M investment in infrastructure.
All BBCE partners and Advisory board members attended BBCE's kick-off meeting at the Riga Technical University (RTU), Latvia. At the official opening ceremony, the participants were honored by the presence and welcome speech from his excellency President of Latvia Egils Levits.

BBCE's opening ceremony at the presence of his excellency President of Latvia Egils Levits.

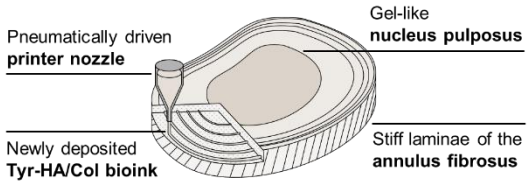
Fund: EU H2020 grant agreement No 857287; ARI Funding 1.4 M CHF; period: 2020 – 2026.

Pres:
3rd INTERREG – 8th TERMIS Winterschool 2020 "Musculoskeletal Tissue Regeneration: From Mechanobiology to *In vitro*/*In vivo* Models and Advanced Imaging". Poster presentation: "Establishment of Baltic Biomaterials Centre of Excellence". Arita Dubnika, Dagnija Loca, Maija Dambrova, Ilze Salma, Konstantins Logviss, Mauro Alini, Aldo R Boccaccini, Janis Locs.
11TH WORLD BIOMATERIALS CONGRESS held online. Poster presentation: "Establishment of the Baltic Biomaterials Centre of Excellence". J Locs, M Dambrova, I Salma, K Logviss, M Alini, M D'Este, AR Boccaccini, L Liverani, D Loca, A Dubnika.

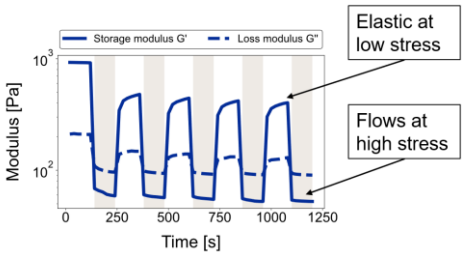
Partners:
- Riga Technical University Rudolfs Cimdins Riga Biomaterials innovations and development centre (RTU RBIDC)
- Latvian Institute of Organic Synthesis
- Riga Stradins University
- Riga Stradins University Institute of Stomatology
- The Institute of Biomaterials at the Department of Materials Science and Engineering of the University of Erlangen-Nuremberg

Engineered full-organ 3D intervertebral disc as standardized model for studying disc degeneration and disease (INDEED) (Ongoing) (M D'Este)

Background: Disc degeneration is a major source of pain and disability for patients worldwide, and a significant financial burden on healthcare providers. Attempts at understanding and addressing this issue are limited by the lack of a suitable model, recapitulating the intrinsic features of the intervertebral disc (IVD). Complex, heterogeneously composed, and subjected to a harsh mechanical environment, the IVD is a challenging organ to replicate. Conventional *in vitro* models, such as 2D and 3D cell cultures, are oversimplifications, which fail to reproduce its composition and organization, and are unable to capture its mechanical properties. The use of explanted human IVDs is rarely an option, owing to their scarcity, comorbidities, and significant donor variability. Animal discs are commonly employed as more accessible alternatives; however, they too display wide biological variability and important biological, compositional, and biomechanical differences compared to human IVDs, which limits their usefulness.
Goal: The overall aim of the project is to use biofabrication to create a tissue-engineered, reproducible, and adaptable 3-dimensional (3D) IVD models outperforming state of the art options for studying IVD disorders; the know-how generated will be a step towards biofabrication of IVD tissue replacements.



Schematic of the 3D bioprinting of an intervertebral disc model



Rheological characterization of INDEED biomaterial ink for its elastic recovery after application of high shear. This test reproduces the conditions experienced during printing.

Results: New composites prepared from a tyramine-derivative of hyaluronan (THA) and unmodified collagen were investigated to develop a bio-ink for 3D printing of the IVD model. The composites undergo gelation via collagen self-assembling, enzymatic oxidation, or visible-light triggered crosslinking with preservation of collagen fibrillogenesis. One of the main challenges of the project is increasing collagen and hyaluronan concentrations approaching physiological values. Our preliminary experiments demonstrate that it possible to obtain biomaterial inks with collagen concentration up to 3%. The biomaterial inks were characterized for their extrusion and shape retention properties (figure) illustrating flow behavior under high shear conditions, like during extrusion from a nozzle, and elastic shape retention at rest. The next phase of the project with printing cell-laden constructs is ongoing.
Fund: SNF 310030E_189310; ARI funding CHF 377'000; Period: 2020 – 2024.

Pres:
G Miklosic, D Eglin, M D'Este. Towards a reproducible intervertebral disc model – a bioprintable nucleus pulposus-like material, Graubünden forscht The Young Researchers Convention Academia Raetica, virtual 2020.

Partners:
- Ferguson S (Prof), ETH Zurich, Zurich, Switzerland
- Guicheux J (Prof), University of Nantes, France
- Le Visage C (Prof), University of Nantes, France
- Helary C, Sorbonne University Paris, France

11 Team Members

Director

Richards R Geoff	Prof, Prof, PhD, MSc	01.10.91

Vice Director

Alini Mauro	Prof, PhD	01.07.99

ARI Management

Bentz Ulrich	Dipl Ing HTL Mikrotechnik	01.08.07
Gueorguiev Boyko	Prof, PhD, MSc (01.03.03 – 30.09.09)	01.07.10
Keller Rolf	Technischer Kaufmann	17.06.96
Stoddart Martin	Prof, PhD (01.08.95– 30.09.96)	01.07.05
Wahl Sonia	Dipl DH Ökonomin HFP	01.12.95
Zeiter Stephan	Dr med vet, PhD (01.02.00 – 12.05.02)	01.06.03

ARI Management Plus (Focus Area Leaders)

D'Este Matteo	PhD	01.04.11
Gehweiler Dominic	Dr med	01.03.16
Goudsouzian Nora	BSc	01.02.02
Grad Sibylle	PD, Dr sc nat, PhD	03.08.00
Lanker Urban	Animal Care (Eidg FA[1])	16.06.86
Moriarty Fintan	PhD, BSc	19.03.07
Varga Peter	PhD	04.08.14
Windolf Markus	Dr biol hum Dipl Ing	01.11.04

Scientific & Technical Staff

Arens Daniel	Dr med vet	01.11.07
Armiento Angela	PhD	01.01.16
Badrutt Isabella	Administrative Assistant	16.07.12
Bagnol Romain	PhD Student, MSc	01.10.19
Barblan Claudia	Administrative Assistant (70%)	15.11.10
Barcik Jan	PhD Student, MSc	01.04.17
Basoli Valentina	PhD	01.04.17
Bluvol Mauro	Chemielaborant (Eidg FA[1])	01.06.03
Brazerol Carmen	Animal Care (Eidg FA[1])	01.03.18
Buchholz Tim	med vet	01.04.19
Buschbaum Jan	Dr rer med	01.08.15
Caspar Jan	Poly mechanics	01.01.09
Ciric Daniel	MSc	01.07.20
Ciriello Simona	PhD, Journal Production Editor	12.09.16
Constant Caroline	Dr med vet, MENG	01.08.19
Della Bella Elena	PhD	01.01.18
Devantay Nicolas	MSc (Nanosciences)	02.12.19
Di Luise Nunzia	PhD	15.06.17
Di Marzio Nicola	PhD Student, MSc	01.01.20
Erb Peter	Animal Care (Eidg FA[1])	03.05.93
Ernst Manuela	MSc, Human Movement Science	01.10.11
Escher Carla	Administrative Assistant (40%)	01.01.95
Faoro Loris	Animal Care	01.11.16
Faoro Pierina	Arztgehilfin, Animal Care (Eidg FA[1]) (70%)	01.12.07
Furlong-Jäggi Pamela	Chemikerin FH, BSc (40%)	01.02.04
Furter Andrea	Animal Care (Eidg FA[1])	24.04.06
Guex Géraldine	PhD	01.03.20
Hatt Phelipe	PhD Student, MSc	01.01.20

Hildebrand Maria	MSc (Immunology)	01.01.18
Hofmann-Fliri Ladina	MSc ETH	01.10.09
Hofstee Marloes	PhD Student, MSc	20.11.17
Kasper Hermann	Dipl Technician HF Systemtechnik	01.10.18
Keller-Stoddart Iris	MTL Technician (60%)	21.10.09
Ladner Yann	PhD Student, MSc	01.08.18
Li Zhen	Assistant Prof, PhD	01.08.11
Ma Junxuan	Dr med, PhD	02.03.17
Menzel Ursula	PhD, Dipl Biol	01.07.11
Miklosic Gregor	PhD Student, MSc	01.02.20
Mischler Dominic	Junior Project Leader (06.09.17 - 28.02.18)	01.10.18
Müller Gregor	Lic phil, Librarian (50%)	17.01.05
Müller Reto	Animal Care (Eidg FA[1])	13.11.01
Mys Karen	PhD	01.06.19
Nehrbass Dirk	Dr med vet, FTA Pathol + Toxicopathol	01.10.10
Perren Dominic	Animal Care	01.02.83
Peter Robert	Dipl Laborant HFP	15.09.84
Post Virginia	PhD (60%)	20.09.10
Ristaniemi Aapo	PhD	16.11.20
Schneider Monika	Administrative Assistant (60%)	06.02.06
Schwab Andrea	PhD	01.04.18
Schwyn Ronald	Dipl Medizintechniker HF	01.11.92
Sercovic Amra	PhD	01.09.20
Serra Tiziano	PhD	01.10.16
Siverino Claudia	PhD	01.11.19
Soubrier Astrid	PhD	05.08.19
Spiller Flurin	Technician	01.08.15
Sprecher Christoph	PhD, Dipl Ing FH	01.02.00
Steiner Sandra	PhD	01.01.14
Sumrall Eric	PhD	01.10.19
Thompson Keith	PhD, BSc (Hons), MSc,	26.05.15
van der Heide Daphne	PhD Student, MSc	01.09.20
Varjas Viktor	MSc, Software Engineer	01.01.14
Vernengo Andrea	PhD	01.09.19
Verrier Sophie	Dr sces sc nat	01.08.04
Vivalda Marisa	Administrative Assistant	01.05.03
Wallimann Alexandra	PhD Student, MSc	01.02.18
Zderic Ivan	MSc ETH	01.02.11
Zuncheddu Daniele	PhD Student	01.02.20
Zweifel Erich	European Industrial Engineer EIE	30.11.92

Apprentice

Ambühl David	Apprentice	01.08.20
Bärtschi Cecilia	Apprentice	01.08.18
Hämmerl Nilo	Apprentice Animal Care	01.04.19

[1] Eidg FA = Eidg Fähigkeitsausweis

Medical Research Fellows

Ahmad Paras	Research Fellow (Pakistan)	01.10.19 – 30.09.20
Bärtl Susanne	Research Fellow (Germany)	01.10.20
Chang He	VET Research Fellow (Austria)	01.07.20 – 31.08.20
Cui Shangbin	Research Fellow (China)	07.01.19 – 18.12.20
Dauwe Jan	Research Fellow (Belgium)	01.08.19 – 31.07.20
Gens Lena	VET Research Fellow (Germany)	01.06.20
Gewiess Jan	Research Fellow (Germany)	06.01.20 – 30.09.20
Gomez Sierra Maria Antonia	Research Fellow (Columbia)	04.02.19 – 31.01.20
Hao Wei	Research Fellow (China)	01.10.20
Magrath Walker	Research Fellow (USA)	06.01.20 – 21.06.20
Pastor Torsten	Research Fellow (Germany)	01.11.20
Pugliese Brenna	VET Research Fellow (USA)	01.07.19 – 12.06.20
Sanchez Rosenberg Guillermo	Research Fellow (Guatemala)	30.06.19 – 30.06.20
Schader Jana	Research Fellow (Germany)	01.07.19 – 30.06.20
Schwegler Hella	VET Research Fellow (Germany)	28.09.20
Stefanov Aleksandar	Research Fellow (Bulgaria)	30.09.19 – 31.01.20
Tourbier Céline	Research Fellow Guest (Germany)	19.02.19 – 16.02.20
Vo Mai Thanh	VET Research Fellow (Germany)	28.09.20
Wittmann Charlotte	VET Research Fellow (Germany)	03.01.19 – 30.04.20
Young Katie	Research Fellow (United Kingdom)	21.09.20 – 20.12.20
Zhang Penghui	Research Fellow (China)	20.01.20 – 25.12.20

Internships

Alig Gion	Internship (Switzerland)	01.10.20
Bashardoust Amirsiavosh	Internship (Iran)	19.08.20
Danker Carolin	Internship (Germany)	01.11.19 – 31.05.20
Eglauf Janick	Internship (Switzerland)	01.06.20 – 15.11.20
	Guest Internship	16.11.20
Füllemann Priscilla	Internship (Germany)	01.11.19 – 31.12.20
Häne Surya	Internship (Switzerland)	01.06.20 – 30.09.20
Hasler Johannes	Guest Internship (Liechtenstein)	01.09.19 – 31.03.20
	Internship	01.04.20 – 30.09.20
Hintermann Joseph	Internship (Switzerland)	01.09.20 – 18.12.20
Kluser Nadine	Internship (Switzerland)	01.09.19 – 30.11.19
	Guest Internship	01.12.19 – 31.07.20
Knecht Manuel	Internship (Switzerland)	01.09.20
Li Wenyue	Internship (China)	09.10.20 – 30.04.20
	Internship (China)	03.06.19 – 30.08.19
Nüesch Andrea	Internship (Switzerland)	01.06.20 – 31.08.20
	Guest Internship	01.09.20
Remppis Magdalena	Internship (Germany)	01.09.20
Staubli Flurina	Internship (Switzerland)	01.12.20
	Guest Internship	01.06.20 – 30.11.20
Trinh Win-Hon	Internship (Switzerland)	01.10.20
Wirth Sylvie	Internship (Switzerland)	01.10.20
Yamamoto Taiyo	Internship (Switzerland)	07.10.19 – 30.04.20

VET Student

Chang He	VET Student	09.03.20 – 17.04.20
Reimann Lotta	VET Student	06.01.20 – 08.03.20
Strunk Maja	VET Student	29.06.20 – 28.08.20

Guest Scientists / Students

Acosta Melanie	Internship (Germany) University Furtwangen, Villingen, Germany	01.09.19 – 21.02.20
Ananthanarayanan Preeta	Guest PhD Student University of Turin, Italy	10.01.20 – 31.07.20
Antonacci Paolo	Guest Internship (Italy) Politecnico di Torino, IT	16.11.20 – 15.05.21
Aygün Talita	Internship (Germany) University Furtwangen, Villingen, Germany	01.09.19 – 21.02.20
Brose Teresa	Guest Internship (Germany/USA) Albert-Ludwigs University, Freiburg, Germany	01.03.20 – 28.02.21
Fu Yanan	Guest Internship (China) Beijing University of Chemical Technology, CN	10.11.20 – 30.04.21
Guoliang Chen	Guest PhD Student (China) Sun Yat-sen University, Guangzhou, CN	10.11.20 – 31.10.21
Guo Peng	Guest PhD Student (China) The 7th Affiliated Hospital, Sun Yat-Sen University, Shenzhen, CN	10.11.20 – 31.10.21
Marcello Edera	Guest Student (Switzerland) ETH Zurich	01.09.20 – 18.12.20
Nan Jiang	Guest Scientist (China) Sichuan University	01.04.19 – 30.04.20
Saravi Babak	Guest Student (Germany) University of Freiburg, Germany	01.08.20 – 31.08.21
Sturm Lisa	Guest Internship (Austria) University of Salzburg, AT	01.10.19 – 29.02.20
Takuya Hidaka	Guest Fellow (Japanese) Kyoto University, JP	01.02.20 – 14.04.20
Wesdorp Tim	Guest PhD Student (The Netherlands) Erasmus University Rotterdam	11.10.19 – 28.08.20

Employees left 2020

Baumgartner Yamina	Technician	01.08.20 – 16.10.20
Eglin David	Prof, PhD	01.06.06 – 30.09.20
Kamer Lukas	Dr med, Dr med dent (80%)	21.05.07 – 30.06.20
Lackington William	PhD	02.07.18 – 14.10.20
Monaco Graziana	PhD Student, MSc	02.11.15 – 30.05.20
Noser Hansrudi	PD Dr ès science EPFL	18.10.04 – 30.06.20
Peroglio Marianna	PhD	01.03.09 – 31.08.20
Rotman Stijn	PhD Student, MSc	26.08.16 – 31.12.20
Stenger Valentina	med vet	01.01.19 – 21.02.20
Vainieri Letizia	PhD Student, MSc	01.09.15 – 29.02.20
Wahl Dieter	Dipl techn Werkzeugspezialist HFP	01.11.93 – 31.03.20
Ziadlou Reihane	PhD Student, MSc	01.11.15 – 30.06.20

Guest Presentations at AO Center

Jan 17, 2020 Prof Laura Suter-Dick, team leader cell biology and *in vitro* toxicology from FHNW University of Applied Sciences and Arts Northwestern Switzerland gave a guest presentation with the title: Modelling liver and kidney *in vitro*.

Feb 28, 2020 Prof Julietta Rau from CNR Istituto di struttura della materia, Rome, Italy gave a guest presentation with the titles: 1. Biomimetic and bioactive materials for regenerative medicine. 2. Raman based diagnostics of tissue pathologies based on biochemical profile.

March 09, 2020 Gabriela Kojonsaari, Clinical Trial Manager and Antti Ritvanen, Product Development from Synoste Ltd., Helsinki, Finland gave a guest presentation with the title: Use of smart materials in intramedullary limb lengthening.

12 ARI Patents

Cannula
- First Application: PCT/CH2008/000238 filed 2008-05-27
- Case: 10.2283
- Developer / Inventors: AOR&D, A Gisep, V Boner, N Suhm

Cannula and Device for Liquid Jet Irrigation of Bone
- First Application: PCT/CH2008/000019 filed 2008-01-15
- Case: 10.2356
- Developer / Inventors: AOR&D, A Gisep, P Kuhn

Bone Fixation Device with Cover
- First Application: PCT/CH2009/000095 filed 2009-03-18
- Case: 10.2406
- Developer / Inventors: AOR&D, RG Richards, C Nötzli

Bone Fixation Device
- First Application: PCT/CH2008/000349 filed 2008-08-15
- Case: 10.2470
- Developer / Inventor: ARI, M Windolf

Device for Processing and Transmitting Measured Signals for Monitoring and/or Controlling Medical Implants, Diagnostic Devices or Biological Processes
- First Application: PCT/CH2009/000198 filed 2009-06-11
- Case: 10.2555
- Developer / Inventor: ARI, M Windolf

Cannula and Kit for Bone Cement Injection
- First Application: PCT/CH2011/000007 filed 2011-04-19
- Case: 10.2567
- Developer / Inventor: ARI, M Windolf

Method for Designing and/or Optimizing a Surgical Device
- First Application: PCT/CH2010/000046 filed 2010-02-25
- Case: 10.2607
- Developer / Inventors: AOR&D, S Brianza, D Schuima, A Tami

Surgical Instrument
- First Application: PCT/CH2010/000330 filed 2010-12-24
- Case: 10.2676
- Developer / Inventors: AOR&D, S Brianza, R Schwyn

Identification and Selection of Functionally Committed Mesenchymal Stem Cells Subpopulations
- First Application: PCT/CH2006/000425 filed 2006-08-11
- Case: 22.2277
- Developer / Inventors: ARI, M Alini, M Stoddart

Method and Device for Measuring the Local Mechanical Resistance of a Porous Body
- First Application: PCT/CH2006/000611 filed 2006-10-31
- Case: 10.2281
- Developer / Inventors: AOR&D, R Schwyn, M Hänni, N Suhm

Thermosensitive Hyaluronic Acid Conjugates and Methods for the Preparation thereof
- First Application: IP 5003 PCT E filed 2013-10-02
- Case: 10.F5003
- Developer / Inventors: AOR&D, M D'Este, D Eglin

Method for manufacturing an auxiliary device suitable for the manufacture of a patient customized implant
- First Application: PCT/CH2015/000001 filed 2015-01-13
- Case: 10.3180
- Developer / Inventors: L Kamer, D Eglin

Kit for assembling a medical device provided with data acquisition means
- First Application: PCT/CH2015/000062 filed 2015-04-29
- Case: 10.3211
- Developer / Inventors: M Windolf

Bone plate
- First Application: PCT/ CH2015/000117 filed 2015-08-07
- Case: 10.3302
- Developer / Inventors: M Windolf, D Epari, M Schütz, T Pohlemann, C Nötzli

Bone Implant for Correcting Unbalanced Growth Plate Activity
- First Application: CH2016/01338 filed 2016-10-06
- Case: 10.3487
- Developer / Inventors: M Windolf, M Schütz

Surface Acoustic Wave (SAW) 3D Printing Method
- First Application: CH01058/17 filed 2017-08-25
- Case: 10.F5004
- Developer / Inventors: T Serra, D Eglin, M Alini

Device and Method for Real-Time Tracking, Navigation and Manipulation of Bone Fragment, Surgical Instruments, Tools or Implants in Computer-Assisted Surgery ("X-in-1 GO")
- First Application: CH00145/18 filed 2018-02-07
- Case: 10.3567
- Developer / Inventor: J Buschbaum, M Windolf

Identification and isolation of osteoprogenitor cells (TGFb Receptor)
- First Application: EP19184241.8 filed 2019-07-03
- Case: F5969
- Developer / Inventors: M Stoddart

Patterning device for the preparation of three-dimensional structures (3D SIM Device)
- First Application: EP20190203370 filed 2019-10-15
- Case: BFHTI-4-EP
- Developer / Inventors: T Serra, M Thurner

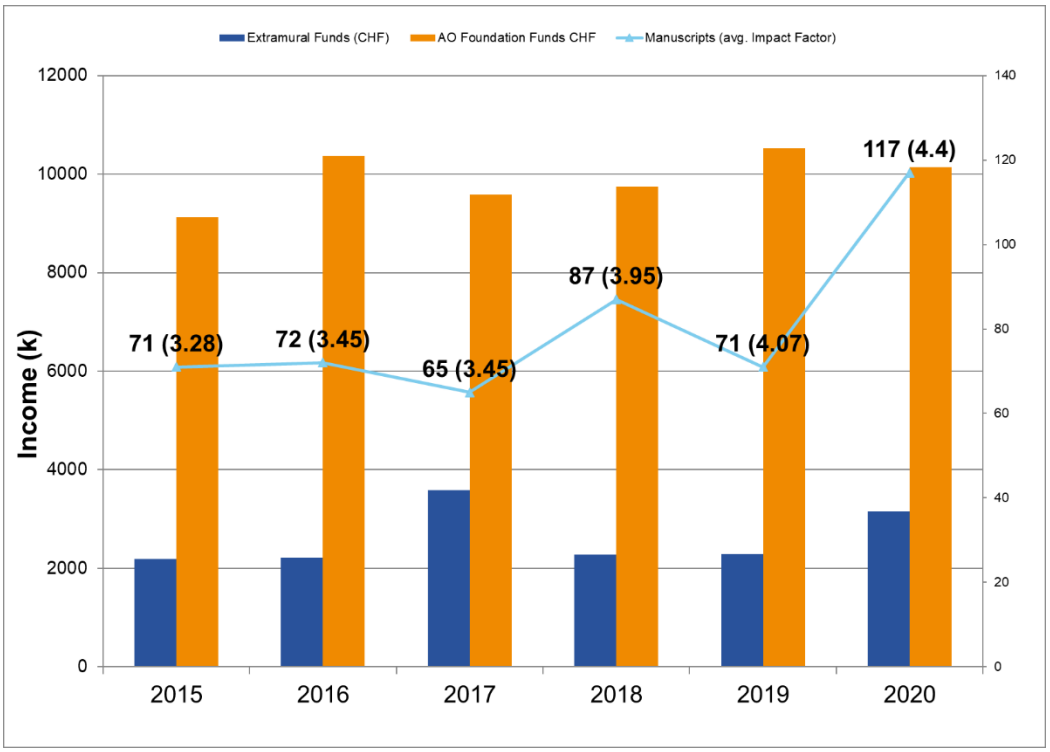
Device for measuring, processing and transmitting implant parameters (Fracture Monitor III)
- First Application: CH01335/19 filed 2019-10-22
- Case: 10.3988
- Developer / Inventors: M Windolf

Biphasic Plate (Biphasic Plate II)
- First Application: CH 01515/19 filed 2019-11-29
- Case: 10.4024
- Developer / Inventors: M Windolf, D Epari

None-stick antibiotics gels (GEDAI gel)
- First Application: CH 01628/19 filed 2019-12-16
- Case: F6183
- Developer / Inventors: M D'Este

13 Publications & Presentations

13.1 2015-2020 Six-year ARI Key Performance Indicators



13.2 2019 Published peer reviewed papers (epub & in print)

Ahmad P, Arshad AI, Della Bella E, Khurshid Z, Stoddart M. Systemic Manifestations of the Periodontal Disease: A Bibliometric Review. Molecules. 2020;25:4508

Ahmad P, Della Bella E, Stoddart MJ. Applications of Bone Morphogenetic Proteins in Dentistry: A Bibliometric Analysis. Biomed Res Int. 2020;2020:5971268

Ahrend MD, Finger F, Grünwald L, Keller G, Baumgartner H. Improving the accuracy of patient positioning for long-leg radiographs using a Taylor Spatial Frame mounted rotation rod. Arch Orthop Trauma Surg. 2020;epub May 6

Ahrend MD, Aurich M, Becher C, Ateschrang A, Schröter S, Walther M, Gottschalk O, Plaass C, Ettinger S, Zinser W, Körner D. Preexisting and treated concomitant ankle instability does not compromise patient-reported outcomes of solitary osteochondral lesions of the talus treated with matrix-induced bone marrow stimulation in the first postoperative year: data from the German Cartilage Registry (KnorpelRegister DGOU). Knee Surg Sports Traumatol Arthrosc. 2020;epub Jul 31

Alexeev D, Cui S, Grad S, Li Z, Ferguson SJ. Mechanical and biological characterization of a composite annulus fibrosus repair strategy in an endplate delamination model. JOR SPINE. 2020;3:e1107

Antunes BP, Vainieri ML, Alini M, Monsonego-Ornan E, Grad S, Yayon A. Enhanced chondrogenic phenotype of primary bovine articular chondrocytes in Fibrin-Hyaluronan hydrogel by multi-axial mechanical loading and FGF18. Acta Biomater. 2020;105:170-179

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Armiento AR, Hatt LP, Sanchez Rosenberg G, Thompson K, Stoddart MJ. Functional Biomaterials for Bone Regeneration: A Lesson in Complex Biology. Adv Funct Mater. 2020;30:1909874

Barcik J, Ernst M, Schwyn R, Freitag L, Dlaska CE, Drenchev L, Todorov S, Skulev H, Epari DR, Zeiter S, Gueorguiev B. Development of surgical tools and procedures for experimental preclinical surgery using computer simulations and 3D printing. iJOE. 2020;16(9):183-95

Behrendt P, Ladner Y, Stoddart MJ, Lippross S, Alini M, Eglin D, Armiento AR. Articular Joint-Simulating Mechanical Load Activates Endogenous TGF-beta in a Highly Cellularized Bioadhesive Hydrogel for Cartilage Repair. Am J Sports Med. 2020;48:210-21

Berset CM, Lanker U, Zeiter S. Survey on sheep usage in biomedical research. Animals. 2020;10:1528

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Buwalda S, Rotman S, Eglin D, Moriarty TF, Bethry A, Garric X, Guillaume O, Nottelet B. Synergistic anti-fouling and bactericidal poly(ether ether ketone) surfaces via a one-step photomodification. Mater Sci Eng C Mater Biol Appl. 2020;111:110811

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Dauwe J, Walters G, Holzer LA, Vanhaecht K, Nijs S. Failure after proximal humeral fracture osteosynthesis: a one-year analysis of hospital-related healthcare cost. Int Orthop. 2020;44:1217-1221

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13.5 Books / Book chapters

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Stoddart M, Richards RG. Growth factors for craniomaxillofacial applications. In: Ehrenfeld M, Futran ND, Manson PN, Prein J (editors). Advanced Craniomaxillofacial Surgery. Tumor, Corrective Bone Surgery and Trauma. Davos: AO Foundation / Thieme; 2020. p. 68-79

13.6 Theses / Dissertations

Buchholz T. Transdermal Fentanyl uptake at two different patch locations in Swiss white alpine sheep.2020 Universität Bern (C. Spadadvecchia, S. Zeiter) – DVM / Dr med vet

Constant C. 2019 Mississippi State University (Babski-Reeves K)- MENG

Constant C. 2020 Residency in Large Animal surgery (Major in Farm Animal Surgery) Research Project: Bovine autologous platelet concentrate: production, hematologic classification and *in vitro* biologic characterization (Desrochers A, Nichols S)- DACVS -LA (Diplomate, American College of Veterinary Surgeons – Large Animal)

Constant C. 2020 Rupture of cranial cruciate ligament in cattle and creation of an intra-articular prosthesis (Nichols S, Desrochers A)- MSc. Graduated on Rector's Honor List with exceptional mention

Häne SI. Treating Discogenic Pain by Reducing Dorsal Root Ganglion Cell Sensitization using the COX-2 Inhibitor Celecoxib – An *in vitro* Study with Inflammatory Cytokine Treated Annulus Fibrosus Cells.2020 ETH Zürich (Z. Li, J. Ma) – MSc ETH

Hasler J. Identification of a suitable RNA extraction method and stable reference genes for qRT-PCR in 3D osteogenic cultures of mesenchymal stromal cells2020 ETH Zürich (MJ. Stoddart, A.R. Armiento, SJ. Ferguson) - MSc ETH HST

Heizmann F. Preclinical testing of TNF-α inhibitor Etanercept in a bovine organ culture model of active discopathy.
2020 Albert-Ludwigs-Universität, Freiburg i. Br. (G. Lang, S. Grad, Z. Li) – MD / Dr.med.

Ivanov S. Minimally invasive surgery in displaced intraarticular fractures of the calcaneus.2020 Medical University of Varna (I. Raykov, B. Gueorguiev) – PhD

Kluser N. 3D Printed Multi-Scale Scaffolds with Topographical Guidance for Annulus Fibrosus Regeneration.2020 ETH Zürich (A. Vernengo, S. Grad, S. Ferguson) – MSc ETH HST

Nowicki BH. Medication related osteonecrosis of the jaw in minipig model: parameters to develop a macroscopic, radiologic and microscopic grading scheme.2020 Universität Zürich (P. Kircher, S. Zeiter) – DVM / Dr med vet

Rotman SG. Biomaterial approaches for the treatment and prevention of orthopaedic infections.2020 University of Twente (D.W. Grijpma, D. Eglin) - PhD

Wangler S. Stem cell homing towards degenerative intervertebral discs: characterization of the migrating subpopulation and its regenerative potential.2020 Universität Bern (M. Alini, L.M. Benneker, M. Peroglio) – MD & PhD

Wystrach LWG. Das Renin-Angiotensin-System der humanen Bandscheibe. 2020 Albert-Ludwigs-Universität Freiburg i. Br. (G. Lang, S. Grad) – MD / Dr med

Ziadlou R. Biological therapy and tissue engineering approaches for the treatment of osteoarthritis.2020 Universität Basel (I. Martin, S. Grad) – PhD

13.7 Abstracts published in journals

Arand C, Wagner D, Kamer L, Noser H, Richards RG, Rommens PM. Correlation between pelvic incidence and acetabular orientation in anteversion and inclination—an analysis based on a 3D statistical model of the pelvic ring. Eur J Trauma Emerg Surg. 2020;46(S1):S226 (ECTES congress called off / abstract published, oral)

Arand C, Wagner D, Kamer L, Noser H, Richards RG, Rommens PM. Korrelation zwischen Pelvic Incidence und räumlicher Orientierung des Acetabulums - eine Analyse basierend auf einem 3D statistischen Modell des Beckenrings. Z Orthop Unfall. 2020;158(S01):S98-S99 (DKOU congress called off / abstract published, oral)

Breceda A, Zderic I, Schopper C, Schader J, Gehweiler D, Richards RG, Gueorguiev B, Sands A. Is the risk of secondary peri-implant fracture after trochanteric fracture consolidation similar when using short or long TFNA? Swiss Med Wkly. 2020;150(Suppl 244):38 (swiss orthop e-congress / oral)

Burkhard B, Schopper C, Ciric D, Mischler D, Gueorguiev B, Varga P. Cyclic perforation risk is increased by overdrilling in locked plating of complex proximal humerus fractures. Bone Joint J. 2020;102-B(Suppl. 11 / Orthop Proc): 75 (EORS virtual, oral)

Dauwe J, Mys K, Putzeys G, Schader J, Richards RG, Gueorguiev B, Varga P, Nijs S. Three-dimensional segmented CT images help orthopedic surgeons and residents to correctly classify proximal humeral fractures. Z Orthop Unfall. 2020;158(S01):S66-S67 (DKOU congress called off / abstract published, oral)

Evers J, Fischer M, Milstrey A, Riesenbeck O, Gehweiler D, Gueorguiev-Rüegg B, Raschke MJ, Ochman S. Computertomographische Stabilitäts-Untersuchung im trimalleolären Sprunggelenksfrakturmodell unter axialer Last. Z Orthop Unfall. 2020;158(S01):S125-S126 (DKOU congress called off / abstract published, oral)

Gueorguiev B, Pukalski Y, Barcik J, Zderic I, Yanev P, Rashkov M, Baltov A, Enchev D. Does coronoid process replacement with individually designed 3D printed prosthesis provide superior stability over grafted reconstruction and screw fixation? A biomechanical study. Z Orthop Unfall. 2020;158(S01):S122-S123 (DKOU congress called off / abstract published, oral)

Gueorguiev B, Zderic I, Blauth M, Weber A, Koch R, Dauwe J, Schader J, Stoffel K, Finkemeier C, Hessmann M. Angular stable locking in a novel intramedullary nail improves construct stability in a distal tibia fracture model. Bone Joint J. 2020;102-B(Suppl. 11 / Orthop Proc):27 (EORS virtual, oral)

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Keck K, Schopper C, Beeres F, Link BC, Babst R, Nebelung S, Gueorguiev B, Knobe M. Innovative Fixierungssysteme bei proximalen Femurfrakturen: Sind Klingen-Schrauben-Kombinationen die Zukunft? Z Orthop Unfall. 2020;158(S01):S109 (DKOU congress called off / abstract published, oral)

Krause F, Zderic I, Seidel A, Gueorguiev B, Attinger MC, Foesel A. Ankle joint pressure in supination-external rotation injuries: A dynamic biomechanic cadaveric study. Foot Ankle Orthop. 2020;5(4) (AOFAS virtual / poster)

Lodde MF, Katthagen JC, Zderic I, Schopper C, Gueorguiev-Rüegg B, Riesenbeck O, Raschke MJ, Hartensuer R. Biomechanische Untersuchung posteriorer Fixierungsmethoden von C1.3 Beckenringfrakturen. Z Orthop Unfall. 2020;158(S01):S225 (DKOU congress called off / abstract published, oral)

Lodde MF, Katthagen JC, Zderic I, Schopper C, Gueorguiev-Rüegg B, Riesenbeck O, Raschke MJ, Hartensuer R. Biomechanischer Vergleich gängiger Techniken zur Stabilisierung des Beckenrings bei Insuffizienzfrakturen. Z Orthop Unfall. 2020;158(S01):S221-S222 (DKOU congress called off / abstract published, oral)

Lodde MF, Katthagen JC, Zderic I, Schopper C, Gueorguiev-Rüegg B, Riesenbeck O, Raschke MJ, Hartensuer R. Biomechanische Untersuchung der transpubischen Schraubenosteosynthese und der anterioren Plattenosteosynthese bei Schmetterlingsfrakturen des Beckenrings. Z Orthop Unfall. 2020;158(S01):S229-S230 (DKOU congress called off / abstract published, poster)

Makelov B, Gueorguiev B, Apivatthakakul T. Can unstable proximal tibial fractures with soft tissue injury be successfully treated with external locked plating? Bone Joint J. 2020;102-B(Suppl. 11 / Orthop Proc):49 (EORS virtual, oral)

Nehrbass D, Cheney D, Gehweiler D, Hearn J, Hildebrand M, Mukhopadhaya J, Narayanan U, Schmittenbecher P, Sepulveda-Oviedo M, Slongo T, Zeiter S, Gueorguiev B, Dwyer JSM. Device-induced Growth Plate Lesions. Classic Examples in Toxicologic Pathology XXVII, 2020 (oral)

Panagiotopoulou V, Ovesy M, Gueorguiev B, Richards RG, Zysset P, Varga P. Micro finite element simulations accurately predict perforation of single screws in the proximal humerus. Bone Joint J. 2020;102-B(Supp. 11 / Orthop Proc):65 (EORS virtual, oral)

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Riesenbeck O, Schulze M, Gehweiler D, Raschke MJ, Hartensuer R. Eine Methode zur Messung der intradiskalen Druckverteilung und des Footprints intervertebraler Implantate - Möglichkeiten und Limitationen. Z Orthop Unfall. 2020;158(S01):S190-S191 (DKOU congress called off / abstract published, oral)

Russo F, Ambrosio L, Peroglio M, Wangler S, Guo W, Grad S, Alini M, Vadalà G, Papalia R, Denaro V. Human mesenchymal stem cells encapsulated in hyaluronic acid and platelet-rich plasma modulate matrix synthesis *ex vivo*. Bone Joint J. 2020;102-B(Suppl. 11 / Orthop Proc):95 (EORS virtual, poster)

Schopper C, Keck K, Beeres F, Link B-C, Babst R, Nebelung S, Zderic I, Gueorguiev B, Knobe M. Screw-blade fixation systems in proximal femur fractures: a biomechanical evaluation. Swiss Med Wkly. 2020;150(Suppl 244):23 (swiss orthop e-congress / oral)

Schwab A, Alini M, Eglin D, D'Este M.3D Drucken von Kollagenfibrillen mit Kontrolle über deren Orientierung in einer Hyaluronsäure Matrix als biomimetisches Knorpelimplantat. Z Orthop Unfall. 2020;158(S01):S220-S221 (DKOU congress called off / abstract published, oral)

Schwab A, Staubli F, Alini M, Eglin D, D'Este M. Einfluss der anisotropen Faserausrichtung und des Kollagen Typs und auf das chondrogene Differenzierungsverhalten. Z Orthop Unfall. 2020;158(S01):S20 (DKOU congress called off / abstract published, poster)

Stefanov A, Ivanov S, Zderic I, Baltov A, Rashkov M, Gehweiler D, Richards RG, Gueorguiev B, Enchev D. Variable-angle locked plating versus interlocked nailing for intraarticular calcaneal fractures – a biomechanical study. Bone Joint J. 2020;102-B(Suppl. 11 / Orthop Proc):85 (EORS virtual, oral)

Tits A, Varga P, Kaux J-F, Plougonven E, Fernandez J, Drion P, Van Lenthe GH, Ruffoni D. Local adaptation of bone micro-structure and canal network to tendon insertion investigated by image-based micro-FE simulations. Bone Reports. 2020;13(Suppl):100393 (ECTS digital / poster)

Wystrach L, Pfannkuche J, Li Z, Grad S, Kubosch DC, Schmal H, Alini M, Lang G. Die protektive Wirkung von Losartan auf humane Nucleus Pulposus Zellen. Z Orthop Unfall. 2020;158(S01):S89-S90 (DKOU congress called off / abstract published, oral)

Zderic I, Schopper C, Lodde M, Wagner D, Richards RG, Gueorguiev B, Rommens P, Acklin Y. A new concept for screw-in-screw fixation of fragility sacrum fractures – biomechanical comparison versus transsacral and SI screw fixations. Swiss Med Wkly. 2020;150(Suppl 244):39-40 (swiss orthop e-congress / oral)

Zderic I, Breceda A, Schopper C, Schader J, Gehweiler D, Richards RG, Gueorguiev B, Sands A. Is the risk of secondary peri-implant fracture after trochanteric consolidation similar when using short or long TFNA? Bone Joint J. 2020;102-B(Suppl. 11 / Orthop Proc):82 (EORS virtual, oral)

Zderic I, Schopper C, Wagner D, Gueorguiev B, Rommens P, Acklin Y. Screw-in-screw fixation of fragility sacrum fractures provides high stability without loosening: A biomechanical study. Bone Joint J. 2020;102-B(Suppl. 11 / Orthop Proc):81 (EORS virtual, oral)

13.8 Abstracts (conference participations)

Ananthanarayanan P, Di Marzio N, Guex AG, Alini M, Serra T, Riganti C. Novel acoustic wave programmed 3D vascularised cancer model in malignant pleural mesothelioma: A platform for drug screening. 2020 Symposium STRATAGEM CA online (oral)

Armiento AR, Hatt LP, Müller WEG, Thompson K, Stoddart MJ. Polyphosphate Nanoparticles Act as an Effective Inorganic Phosphate Source during Osteogenic Differentiation of BM-MSCs. 2020 ORS (poster)

Bagnol R, Sprecher CM, Peroglio M, Richards RG, Eglin D. 3D printing of a calcium phosphate paste for the production of patient specific bone substitutes. 2020 GR forscht virtual (oral)

Bagnol R, Sprecher CM, Peroglio M, Mahou M, Buchler P, Lieger O, Richards RG, Eglin D. Improving shape stability of patient-specific calcium phosphate bone substitutes using co-axial 3D printing. 2020 WBC virtual (poster)

Barcik J, Ernst M, Balligand M, Dlaska CE, Drenchev L, Todorov S, Gueorguiev B, Skulev H, Zeiter S, Epari D, Windolf M. Continuous monitoring of bone healing by measurement of fracture callus stiffness. 2020 GR forscht virtual (poster)

Basoli V, Rothweiler R, Johnstone B, Alini M, Stoddart MJ. Predicting and promoting human MSC chondrogenesis by way of TGFβ receptor profiles: Towards personalized medicine. 2020 ORS (poster)

Cui S, Zhou Z, Alini M, Grad S, Li Z. High impact loading organ culture model to investigate the post-traumatic disc degenerative condition. 2020 GR forscht virtual (oral)

Della Bella E, Menzel U, Stoddart MJ. Dexamethasone regulates circular RNA expression in early osteogenesis and chondrogenesis of human bone marrow mesenchymal stromal cells. 2020 ORS (oral)

Di Marzio N, Richards RG, Alini M, Eglin D, Serra T. Acoustic waves cell patterning for spatially orchestrated vasculature system in tissue engineering. 2020 SSB+RM YSS (oral)

Dubnika A, Loca D, Dambrova M, Salma I, Logviss K, Alini M, Boccaccini AR, Locs J. Establishment of Baltic Biomaterials Centre of Excellence2020 INTERREG-TERMIS Winterschool (poster)

Fletcher JWA, Neumann V, Silva J, Mys K, Panagiotopoulou VC, Verschueren A, Gueorguiev B, Richards RG, Whitehouse M, Preatoni E, Gill H. TightRight: augmenting screwdrivers to reduce bone stripping rates and optimise tightness when inserting non-locking screws. 2020 ORS (poster / ICORS best posters)

Gewiess J, Ma J, Richards RG, Grad S, Alini M, Peroglio M. The missing link: From mechanical intervertebral disc overloading to low back pain.2020 GR forscht virtual (poster)

Häckel S, Häne S, Ma J, Li Z, Pfannkuche J, Peroglio M, Hoppe S, Benneker L, Lang G, Südkamp N, Grad S. Could the COX-2 inhibitor Celecoxib influence discogenic pain? An *in vitro* study with inflamed annulus fibrosus cells. 2020 EFORT virtual (poster)

Hatt LP, Thompson K, Müller WEG, Stoddart MJ, Armiento AR. Calcium Polyphosphate Nanoparticles Act as an Effective Inorganic Phosphate Source during the *in vitro* Osteogenic Differentiation of Human Mesenchymal Stem Cells. 2020 SSB+RM YSS (oral)

Hofstee M, Riool M, Terjajevs I, Thompson K, Stoddart M, Zaat SA, Moriarty TF. 3-dimensional *in vitro* Staphylococcus aureus abscess communities display antibiotic tolerance and protection from neutrophil clearance. 2020 GR forscht virtual (oral)

Ivanov S, Stefanov A, Zderic I, Rodemund C, Schepers T, Richards RG, Raykov D, Gueorguiev B. Analysis of different screw configurations for fixation of Sanders type II B intraarticular calcaneal fractures – a biomechanical human cadaveric study. 2020 EFORT virtual (oral)

Keltz E, Mora AJ, Fletcher J, Gueorguiev B, Keren Y. Orthopedic screws insertion simulation model with real time feedback is efficient in enhancing skill and musculoskeletal memory. 2020 IOA (oral)

Kluser N, Vernengo A, Jansen JU, Neidlinger-Wilke C, Wilke HJ, Li Z, Grad S. Papain-induced *ex vivo* model for intervertebral disc degeneration and the influence of daily physiological loading. 2020 GR forscht virtual (poster)

Kuttner H, Pfister S, Moriarty TF, Aepli M, Meier C, Kalberer F, Wahl P. Eine periprothetische Infektion mit *Actinomyces radingae* könnte zur Identifikation einer missachteten Quelle intraoperativer Kontamination führen. 2020 Endoprothetik (poster)

Lackington W, Hildebrand M, Alini M, Zeiter S, Thompson K. Local delivery of IL-1Ra as a strategy to enhance long bone healing. 2020 ORS (poster)

Lackington W, Gomez M, Vazquez A, O'Brien FJ, Thompson K. Local non-viral gene delivery to immunomodulate and enhance fracture healing. 2020 GR forscht virtual (oral)

Ladner Y, Armiento AR, Snedeker JG, Stoddart MJ. Controlled Factorial Design Of Experiment To Optimize Mechano-induced Chondrogenesis Of Human MSCs – A Feasibility Study. 2020 ORS (poster)

Ladner Y, Armiento AR, Snedeker JG, Stoddart MJ. Full factorial design of experiment elucidates interaction of mechanical parameters driving *in vitro* cartilage formation in a bioreactor. 2020 GR forscht virtual (oral)

Ladner YD, Armiento AR, Snedeker JG, Stoddart MJ. Controlled factorial design of experiment to optimize mechano-induced chondrogenesis of human MSCS. 2020 EORS virtual (oral)

Li Z, Pfannkuche J, Kubosch D, Südkamp N, Alini M, Grad S, Lang G. Angiotensin II receptor antagonist inhibits TNFα induced degeneration process in human nucleus pulposus cells. 2020 ORS (poster)

Locs J, Dambrova M, Salma I, Logviss K, Alini M, D'Este M, Boccaccini AR, Liverani L, Loca D, Dubnika A. Establishment of the Baltic Biomaterials Centre of Excellence. 2020 WBC virtual (poster)

Long RG, Ferguson SJ, Benneker L, Sakai D, Li Z, Pandit A, Grijpma DW, Eglin D, Zeiter S, Schmid T, Eberli U, Nehrbass D, Iatridis JC, Alini M, Grad S. Morphological and Biomechanical Effects of Annulus Fibrosus Injury and Repair in an Ovine Cervical Model. 2020 ORS (poster)

Miklosic G, Eglin D, D'Este M. Towards a reproducible intervertebral disc model – a bioprintable nucleus pulposus-like material. 2020 GR forscht virtual (oral)

Mys K, Varga P, Stockmans F, Gueorguiev B, Neumann V, Wyers CE, van den Bergh JPW, van Lenthe GH. Adaptive local thresholding improves the accuracy of XTremeCT to quantify trabecular structure in the radius. 2020 EORS virtual (oral)

Pfannkuche JJ, Li Z, Grad S, Alini M, Häckel S, Südkamp N, Schmal H, Lang G. Anti-inflammatory effects of Losartan in human nucleus pulposus cells. 2020 Eurospine virtual (poster)

Pukalski Y, Barcik J, Zderic I, Yanev P, Baltov A, Rashkov M, Richards RG, Gueorguiev B, Enchev D. Superior stability of coronoid process replacement achieved via individually designed 3D printed prosthesis with curved cemented intramedullary stem. 2020 ORS (poster)

Pukalski Y, Barcik J, Zderic I, Yanev P, Baltov A, Rashkov M, Richards RG, Gueorguiev B, Enchev D. Individualized coronoid process reconstruction with 3D printed prosthesis provides superior stability over radial head graft reconstruction and coronoid screw fixation. 2020 EFORT virtual (oral)

Rotman SG, Grijpma DW, Post V, Eglin D, Moriarty TF. Local bacteriophage delivery by a thermo-responsive hydrogel system for treatment of medical infections – an *in vitro* assessment. 2020 SSB+RM YSS (poster / rfo)

Rotman S, Grijpma D, Richards RG, Moriarty TF, Eglin D, Guillaume O. Bone targeted antibiotic delivery with functionalized poly (ϵ-caprolactone) microparticles.2020 GR forscht virtual (oral)

Schader JF, Mischler D, Dauwe J, Gueorguiev B, Varga P. Improving stability of proximal humerus fracture locked plating by means of patient-specific computational optimization. 2020 AGA virtual (oral)

Schader JF, Zderic I, Gehweiler D, Dauwe J, Mys K, Danker C, Acklin Y, Gueorguiev B, Stoffel K. Standardized fractures – introducing a novel approach to orthopedic implant testing. 2020 EORS virtual (poster)

Schader JF, Zderic I, Dauwe J, Mys K, Gehweiler D, Acklin Y, Gueorguiev B, Stoffel K. Biomechanical comparison of standardized stable pertrochanteric fractures versus osteotomies for orthopaedic implant testing. 2020 GR forscht virtual (oral)

Schader JF, Helfen T, Braunstein V, Ockert B, Haasters F, Hertel R, Südkamp N, Sprecher CM. Patient-specific aiming device increases precision of guide wire placement in arthritic glenoids – an experimental study. 2020 GR forscht virtual (poster)

Schader JF, Mischler D, Dauwe J, Gueorguiev B, Varga P. Patient-specific computational optimisation of the locking screw orientation of a proximal humerus plate in osteoporotic fracture fixation. 2020 EFORT virtual (oral)

Schol J, Sakai D, Takayuki W, Peroglio M, Li Z, Wangler S, Zeiter S, Grad S, Alini M. Locally Delivered Mesenchymal Stem Cell Homing Enables Intervertebral Disc Repair: A Proof-of-Concept Study. 2020 ORS (poster)

Schwab A, Eglin D, Alini M, D'Este M. Control of microarchitecture within biomaterial ink through 3D printing to guide cell migration. 2020 WBC virtual (oral)

Soubrier A, Grad S, Alini M, I. J, Peroglio M. Unloading of intervertebral disc to improve low back pain management: from organ model to clinics. 2020 GR forscht virtual (oral)

Stefanov A, Ivanov S, Zderic I, Baltov A, Rashkov M, Gehweiler D, Richards RG, Gueorguiev B, Enchev D. Variable-angle locked plating versus interlocked nailing for intraarticular calcaneal fractures – a biomechanical study. 2020 BOTA (poster)

Sumrall E, Wallimann A, Hildebrand M, Zeiter S, Richards RG, Schwarz E, Moriarty TF, Muthukrishnan G. A humanized mouse model to exhibit *Staphyloccocus aureus* osteomyelitis. 2020 OBIC virtual (poster)

Vernengo A, Li Z, Mys K, Varga P, Grad S, Alini M, Eglin D. Annulus Fibrosus Tissue Engineering Using 3D Printed Poly(ε-caprolactone) Scaffolds with Oriented Ply Structure. 2020 ORS (poster)

Wallimann A, Thompson K, Moriarty TF, Akdis C, O'Mahony L. The influence of microbial-derived metabolites on bone health. 2020 EAACI IWS (poster)

Wallimann A, Thompson K, Moriarty TF, Akdis C, O'Mahony L. The influence of short-chain fatty acids on bone health. 2020 GR forscht virtual (oral)

Wallimann A, Thompson K, Puliese B, Magrath W, Moriarty TF, Akdis CA, O'Mahony L. The influence of short-chain fatty acids on bone health.2020 WIRM virtual (oral)

Wesdorp MA, Schwab A, Narcisi R, Eglin D, van Osch GJVM, D'Este M. Assessment of the neutrophil response to a panel of synthetic and natural derived biomaterials; a novel comprehensive *in vitro* approach. 2020 NBTE virtual (oral)

Yamamoto T. Development of an ECM derived tough hydrogel for cartilage tissue engineering. 2020 SSB+RM YSS (poster)

Zhang P, Ziadlou R, Basoli V, Li Z, Grad S. *In vitro* and *ex vivo* models to test the anti-inflammatory and regenerative effects of small molecules in osteoarthritis. 2020 GR forscht virtual (poster)

Ziadlou R, Barbero A, Stoddart MJ, Wirth M, Li Z, Martin I, Wang X, Qin L, Alini M, Grad S. Regulation of Inflammatory Response in Human Osteoarthritic Chondrocytes by Novel Herbal Small Molecules. 2020 SSB+RM YSS (poster)

Zuncheddu D, Generelli S, Kurth F, Serra PA, Rocchitta G, Grad S, Basoli V. Real time quality monitoring of engineered tissue for regenerative medicine. 2020 GR forscht virtual (oral)

13.9 Presentations (not in conference proceedings)

08.09.2020	Richards Geoff: "AO – past, present, future", British Orthopaedic Research Society (BORS), UK (virtual meeting)
19.09.2020	Richards Geoff: "Smart Surgery, the AO Fracture Monitor", Japanese Society for Fracture Repair (JSFR), Japan (virtual meeting)
27.08.2020	Alini Mauro: "Research Ethics", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia
05.11.2020	Alini Mauro: "Stem Cell for Intervertebral Disc Regeneration: How and when!", Webinar series "Regenerative Medicine; Cells & beyond" organized by the Iranian Council for Development of Stem Cell Sciences and Technologies and Tehran Medical University.
02.03.2020	Gueorguiev Boyko: "Why and how do locking plates fail?", AO Trauma India Symposium 'Complex Trauma Management', Agra, India (Invited Speaker)
04.-07.03.2020	Gueorguiev Boyko: "Nonunions – just a biomechanical problem", 6th Serbian Trauma Association Congress, Vernjacka Banja, Serbia (Invited Speaker)
04.-07.03.2020	Gueorguiev Boyko: "Biomechanics and design of intramedullary nails", 6th Serbian Trauma Association Congress, Vernjacka Banja, Serbia (Invited Speaker)

02.-04.10.2020	Gueorguiev Boyko: "Digitally-enhanced surgical training and patient outcome assessment", 24th National Conference of the Bulgarian Orthopedic and Traumatology Association, Plovdiv, Bulgaria (Invited Speaker)
14.-17.12.2020	Gueorguiev Boyko: "Individualized coronoid process reconstruction with 3D printed prosthesis provides superior stability over radial head graft reconstruction and coronoid screw fixation", Hybrid 72nd Annual International Congress of the Egyptian Orthopaedic Association, Cairo, Egypt, (Virtual Invited Speaker)
24.01.2020	Stoddart Martin: "Functional markers of bone marrow derived mesenchymal stromal cells for orthopaedic applications", Phacilitate 2020, Florida, USA (Invited Speaker)
06.03.2020	Stoddart Martin: "Mechano-regulation of stem cell fate for musculoskeletal tissue engineering: Can we repurpose rehabilitation?", International Society of Physical and Rehabilitation Medicine (ISPRM) 2020, Orlando, USA (Invited Speaker)
03.07.2020	Stoddart Martin: "Modeling human diseases in animals. Science Technology and Reconstructive Surgery 2020", Stanford University, California, USA (virtual meeting)
18.08.2020	Stoddart Martin: "Laboratory management, cell culture databases", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia
20.08.2020	Stoddart Martin: "Journal citations, impact factor, h-index, open archive", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia
19.09.2020	Stoddart Martin: "Regulating MSC differentiation by mechanics: Can we repurpose rehabilitation?", Virtual AGA Annual Conference, AGAnywhere
19.09.2020	Stoddart Martin: "From the past to the future: The success story of AO", Virtual AGA Annual Conference, AGAnywhere (Keynote Lecture)
05.12.2020	Stoddart Martin: "Enhanced bone healing–bone substitutes", AO Trauma Online Masters Course–Nonunion and Malunion, Davos, Switzerland
07.02.2020	Zeiter Stephan: "ORS Preclinical Model Section Workshop: Planning, Preparing, Conducting and Reporting of Preclinical Studies", Orthopaedic Research Society (ORS) Annual Meeting, Phoenix, Arizona, USA (Invited Speaker)
13.08.2020	Armiento Angela & Di Luise Nunzia: "Electronic Lab Journal and Experimental Planning", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia
18.08.2020	Bentz Ulrich: "Introduction to ISO 9001", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia
19.08.2020	Bentz Ulrich: "Introduction to ISO 13485:2016", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia

06.08.2020 Gehweiler Dominic: "Scanning with a clinical CT *in vivo & ex vivo*", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia

10.08.2020 Gehweiler Dominic: "Image processing and evaluation", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia

21.08.2020 Goudsouzian Nora: "Histology laboratory management, histology databases, data management for industry projects", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia

05.11.2020 Moriarty Fintan: "Antimicrobial loaded hydrogel for the treatment of chronic implant related MRSA osteomyelitis", Infection and Microbiology Seminar, University Hospital, Zurich, Switzerland (virtual meeting)

07.08.2020 Mys Karen: "Scanning with uCT *in vivo & ex vivo*", virtual external training visit, Baltic Biomaterials Centre of Excellence (BBCE), Riga, Latvia