MERLN Institute

MERLN self-evaluation 2014-2020

Faculty of Health, Medicine and Life Sciences

Maastricht University | Maastricht UMC+

Annex 3:

Narrative case studies

Case study 1:

Biofabrication technologies

Background

Current RM products often suffer from high costs and laborious techniques that complicate scaling-up production. First generation products consisted of cells in suspension, encapsulated in hydrogels, or seeded into 3D porous matrices. These products demonstrated the potential of RM therapies by reducing pain and restoring tissue continuity. Yet, the regenerated tissue is not always as functional as the original one. This leads to degeneration few years after surgery and consequently to the need of another surgery. Causes are different. For example, cells need to be expanded before achieving a sufficient number for implantation. In the body, cell proliferation and homeostasis occur in a 3D environment. In contrast, cell expansion in a laboratory is typically performed on 2D surfaces. This is associated with a loss of the original cell phenotype. Consequently, the expanded cells produce a different extracellular matrix (ECM), ultimately resulting in a tissue formation that is different than the targeted tissue to regenerate.

Biofabrication is a growing interdisciplinary field that finds diverse applications spanning from soft robotics, to 3D *in vitro* models and tissue engineering. Biofabrication in tissue engineering and RM is defined as the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through bioprinting or bioassembly and subsequent tissue maturation processes. Additive manufacturing of 3D scaffolds falls with this strategy when the subsequent maturation process, *in vitro* through cell seeding or *in vivo* through the recruitment of endogenous cells, yields a structural biologically functional construct.

MERLN's approach

At MERLN, biofabrication activities are coordinated by Prof. Lorenzo Moroni, who is the Chair of the Complex Tissue Regeneration Department. He collaborates with various PIs on the use of biofabrication technologies for specific research topics. Dr Carlos Mota is active in bioprinting, development of 3D *in vitro* models, and development of new biofabrication technologies. Dr Matthew Baker contributes with new hydrogel formulations as dynamic bioinks for bioprinting. Dr Paul Wieringa uses various biofabrication techniques for establishing 3D *in vitro* models of peripheral nervous system and innervation of tissues. Dr Stefan Giselbrecht focuses on the integration of biofabrication techniques with microfluidic platforms. Dr Aart van Apeldoorn uses biofabrication technologies for developing methods for transplantation of islet of Langerhans. Prof. Truckenmüller works on bioassembly approaches. Prof. Pamela Habibovic and Prof. Martijn van Griensven use the technology for development of constructs for orthopedic and craniomaxillofacial applications.

Below, several examples of current research lines are given:
Example: Design of scaffolds able to control and steer (stem) cell activity. Stem cells are a fascinating and promising source to regenerate tissues and organs due to their potential to differentiated into multiple specialized cells. Yet, better control over cell-material interactions is necessary to maintain tissue engineered constructs in time. It is crucial to control stem cell quiescence, proliferation and differentiation in 3D scaffolds while maintaining cells viable *in situ*.

Example: Developing current and new biofabrication technologies based on additive manufacturing, bioprinting, bio-assembly, and electrospinning. Among biofabrication technologies, bioprinting, additive manufacturing, bioassembly, and spinning technologies form crucial clusters that shall be used for this purpose. These technologies will be further advanced in the future to include surface engineering methods during fabrication.

Example: Integrating neural and vascular cues in tissue and organ regeneration strategies. Initial investigations on how different biofabrication platforms could be combined to recreate a synthetic mimicry of the ECM of the peripheral nervous system have been started. The goal in the coming years is to complement this know-how with vascularization and understand how neurovascular stimuli can modulate tissue regeneration.

Example: Engineering the immune response of biomaterials, scaffolds, and biomedical devices. Engineered devices with surface properties able to steer the foreign body response to synthesize a vascular graft for dialytic patients have already been successfully created. Further deepening our understanding of how biomedical implants can be engineered to steer the foreign body response is an exciting field in RM as it will improve the integration of biofabricated substitutes with surrounding tissues.

Example: Applying biofabrication technologies to study regenerative and degenerative phenomena. 3D constructs can be used as 3D *in vitro* models to understand biological mechanism behind tissue regeneration, homeostasis, and eventual degeneration. This will be fed back into the design of biofabricated constructs to, on the one side, achieve a better 3D model, and on the other side improve therapies for targeted diseases.

Example: Translating developments directly to the clinical environment. When possible, biofabrication techniques are directly used in the clinical arena for "end-stage" cases. For instance, recently, together with the Department of Trauma Surgery of MUMC (Prof. Martijn Poeze en Dr Taco Blokhuis) a personalized scaffold was made using 3D printing according to a CT scan. This was implanted in a 15 cm tibial defect, loaded with stem cells isolated in the operating room as well as material from a reamer irrigator aspirator procedure (containing bone dust and bone marrow among others). The approach was successful, since after 6 months most of the defect was bridged in a patient for whom all other treatment options were exhausted.

Key points

- We have one of the largest biofabrication centers in Europe with frontier technologies.
- We focus both on the development of new biofabrication technology and application of the existing technology in a variety of (clinical) areas.

Collaborations
Internal:
- Prof. Ron Heeren (UM, M4I) – on new methodologies for tissue characterization
- Dr Florian Caiment (UM, Toxicogenomics) – on applications of bioprinted 3D *in vitro* models in toxicology
- Prof. Tilman Hackeng (UM, Biochemistry) – on development and synthesis of peptides
- Dr Pieter Emans (MUMC+, Orthopedics) – on integrating imaging technologies with 3D printed implants for joint regeneration
- Prof. Peter Kessler (MUMC+, Craniomaxillofacial surgery) – on developing novel implants for treatment of cranial defects
- Prof. Nicole Bouvy (MUMC+, General surgery) – on biomaterials biocompatibility
- Prof. Barend Mees, Prof. Sandro Gelsomino, Prof. Jos Maessen (MUMC+, Vascular and cardiothoracic surgery) – on translating coronary artery and cardiac patch scaffolds to preclinical validation

National:
- Prof. Carlijn Bouten, Prof. Patricia Dankers (Eindhoven University of Technology), Dr Caroline Cheng (University Medical Center Utrecht), Prof. Paul Quax, Dr Joris Rotmans (Leiden University Medical Center) – on smart biomaterials and cardiovascular applications
- Dr Eric Farrell (Erasmus Medical Center) – on vascularized bone models

International:
- Prof. Giuseppe Gigli (CNR Nanotec) – on neurodegenerative and oncology 3D *in vitro* models
- Dr Alessandro Patelli (University of Padova) – on plasma technology
- Prof. Joao Mano (University of Aveiro) – on natural polymers
- Prof. Lies Geris, Prof. Frank Luyten (Catholic University of Leuven) – on 3D bone models
- Prof. Izabela Stancu (University of Bucharest) – on mechano-tomography characterization
- Dr Matteo D'Este (AO Foundation) – on hyaluronic acid formulations for bioprinting

Industry:
- Idonial, Cellink, Poietis and Aspect Biosystems – on additive manufacturing and development of new bioprinting techniques
- Cidetec – on nanomaterials for bioprinting
- PreSens – on biosensors
- Fluicell, TissUse and React4Life – on microfluidic and bioreactor technologies
- Stem Cell technologies – on stem cell tools to facilitate bioprinting

Main grants
Total amount (MERLN part)
- EU-H2020-FETOPEN-1-2016-2017 "B2B" project (2018-2022); € 3,799,371 (€ 464,384)
- EU-H2020-SC1-BHC-2018-2020 "SCREENED" project (2019-2024); € 5,655,088 (€ 1,293,500)
- Regional financing by UM, DSM, Province of Limburg "BMC" project (2016-2020); € 2,400,000 (€ 2,400,000)
- RegMed XB Cardiovascular Moonshot (2020-2021); € 190,949 (€ 190,949)
- EU-H2020-SC1-BHC-07-2019 "JOINTPROMISE" project (2020-2024); € 7,901,115 (€ 1,694,275)
- EU-H2020-SC1-BHC-07-2019 "cmRNAbone" project; € 6,257,759 (€ 999,749)
- EU-H2020-MSC-ITN-2019 "SINERGIA" project (2020-2024); € 3,951,996 (€ 531,240)

Selected publications

- **Mota, C., Camarero-Espinosa, S., Baker, M. B., Wieringa, P., Moroni, L.** (2020). Bioprinting: From Tissue and Organ Development to *in vitro* Models. Chemical Reviews, 120(19), 10547-10607. https://doi.org/10.1021/acs.chemrev.9b00789
- **Zonderland, J., Gomes, D. B., Pallada, Y., Moldero, I. L., Camarero Espinosa, S., Moroni, L.** (2020). Mechanosensitive regulation of stanniocalcin 1 by zyxin and actin myosin in human mesenchymal stromal cells. Stem Cells, 38, 948-959. https://doi.org/10.1002/stem.3198
- **Addario, G.,** Djudjaj, S., Farè, S., Boor, P., **Moroni, L., Mota, C.** (2020). Microfluidic bioprinting towards a renal *in vitro* model. Bioprinting, 20, [e00108]. https://doi.org/10.1016/j.bprint.2020.e00108
- **Malheiro, A., Morgan, F., Baker, M. Moroni, L., Wieringa, P.** (2020). A three-dimensional biomimetic peripheral nerve model for drug testing and disease modelling. Biomaterials, 257, [120230]. https://doi.org/10.1016/j.biomaterials.2020.120230
- **Ooi, H. W., Mota, C.,** ten Cate, A. T., **Calore, A., Moroni, L., Baker, M. B.** (2018). Thiol-Ene Alginate Hydrogels as Versatile Bioinks for Bioprinting. Biomacromolecules, 19(8), 3390-3400. https://doi.org/10.1021/acs.biomac.8b00696

Selected outreach activities
- TV VPRO series "De toekomst is fantastisch", 2019. (https://www.vpro.nl/programmas/de-toekomst-is-fantastisch/kijk/afleveringen/aflevering-6.html)
- CGTN RAZOR series, 2019. (https://newseu.cgtn.com/news/2019-11-26/3D-bio-printing-and-AI-Dementia-detector-RAZOR-full-episode-LUM6SCw3W8/index.html)
- TV report in RTL Editie NL on the clinical translation case described above, 2020. (https://www.rtlnieuws.nl/tech/video/5205347/3d-printer-voorkomt-amputatie)
- Interviews in newspapers: Trouw, Technisch Dagblad, Business Wire, De Limburger, for example in 2018. (https://www.trouw.nl/nieuws/stel-je-voor-een-verloren-been-groeit-vanzelf-aan~b4d095f4/)

Case study 2:

Microtechnology and fluidics for on-chip applications

Background
In the continued endeavour to gain control over the smallest units of life in the human body, the cells, to actively recruit and employ them during processes of repair and regeneration of compromised tissues and organs as a consequence of trauma, disease or genetic disorder, effective tools and technologies (inter)acting at a similar scale are essential. This (cell) scale is the micrometre scale, the technology is termed 'microtechnology'. Microtechnology is the technology of the creation, or 'microfabrication', of structures/patterns and devices at the microscale, and of their applications. A micrometre is one thousandth of a millimetre. Micropatterns/structures are created using technologies as similarly known from the manufacturing of computer chips in semiconductor industry. The central technology in this conjunction is lithography in its meanwhile many variants. For example, a comparatively young representative of this technology, the so-called two-photon laser photolithography, uses a laser that cures structures in a light- or 'photo'-curable liquid resin to create complex three-dimensional structures down to a tenth of a micrometre feature size. An example for micro(scale) devices is/are microfluidic chips, with microfluidics being "the science and technology of systems that process or manipulate small (10^{-9} [one billionth] to 10^{-18} litres) amounts of fluids, using channels with dimensions of tens to hundreds of micrometres" (G. Whitesides, Nature, 2006).

MERLN's approach
At MERLN, within the teams of Dr Stefan Giselbrecht, Prof. Pamela Habibovic, Dr Niloofar Tahmasebi and Prof. Roman Truckenmüller, we apply microtechnologies and fluidics in the field of tissue regeneration. We use microfabrication to create next-generation surfaces of implants from biomaterials, such as cell or tissue 'scaffolds' to replace lost, damaged

or diseased tissues together with the patient's own (stem) cells. Thereby, we aim for improvement of the implants' long-term functional integration into and interaction with the surrounding healthy tissues. Condensed in microfabricated, miniaturized arrayed libraries, to which human cells can be exposed, we can screen hundreds to thousands of these materials/material surfaces to find the best candidate for a certain clinical application. We also use microfabrication and fluidics to create micro(sized) biomaterials that are provided with properties to support cellular function. One example for the application of the microbiomaterials in the form of (micro)engineered micro-objects pioneered by us is in engineered tissue replacements created by aggregating/clustering them in multiple steps with patient cells. Such a multistep approach to the assembly of tissues from the ground up from cells via micro tissue building blocks to meso- and macroscale tissues is called 'bottom-up tissue engineering'. We further use microfabrication and fluidics for so-called 'organ on chips', in which we grow human cells on porous membranes or embedded in highly hydrated macromolecular networks imitating the extracellular space. Thereby, the culture medium is flown through the chip, similar to blood in our vasculature. While such systems as mimics of healthy or diseased human organs so far have been developed as models for fundamental academic research and for the future testing of the toxicity and efficacy of pharmaceutical compounds, MERLN pioneered their use for the assessment/evaluation of biomaterials. Last but not least, we use microfabrication to create advanced micro or mini culture dishes or wells in which we allow stem cells to self-assemble and organize into three-dimensional microtissues with complex outer shape and inner architecture, and, thereby mimicking stages of embryonic development, into artificial/synthetic (micro) organs, termed 'organoids'. These microtissues can be used to create substitute tissues from the bottom up and to increase our understanding how developmental processes can be exploited for tomorrow's tissue regeneration therapies and treatments, respectively.

Key points

- We have a well-equipped laboratory, including a clean room, to do most of our micro-technology and -fluidics research.
- We use our microtechnology- and fluidics platform for developing organ/disease-on-chip models, for high-throughput production and screening of biomaterials, for high-throughput 3D cell culture and for bottom-up tissue engineered constructs.

Collaborations

Internal:

- Prof. Leon Schurgers (UM, Biochemistry) and Dr Dr Barend Mees (MUMC+, Vascular surgery) – on an *in vitro* model of coronary artery calcification
- Dr Niki Reynaert (UM, Pulmonology) – on bronchial organoid engineering
- Prof. Coen Stehouwer and Prof. Casper Schalkwijk, (MUMC+, Internal medicine) – on an *in vitro* model of the metabolic syndrome

National:

- Prof. Pieter Hiemstra (Leiden University Medical Center) – on *in vitro* models of the lung (alveoli and bronchioles)
- Dr Robbert Rottier (Erasmus University Medical Center) – on *in vitro* models of a corona virus infection of the respiratory tract
- Prof. Marianne Verhaar (University Medical Center Utrecht) – on kidney organoid/'tubuloid' engineering
- Prof. Roos Masereeuw (Utrecht University) – on *in vitro* models of the kidney (proximal tubule)
- Prof. Jolanda Kluin (Amsterdam University Medical Center) – on 3D culture of aortic valve leaflet interstitial cells in microwell arrays

Main grants

Total amount (MERLN part)

- ZonMw COVID-19 MKMD - Lung bioreactor; Employing a physiological microfluidic lung bioreactor to improve understanding of SARS-CoV2 biology and testing of therapeutics (2020-2022); € 530,000 (€ 170,734)
- Interreg Vlaanderen-Nederland – Biomat; Biomat on microfluidic chip (2018-2021); € 4,500,000 (€ 1,837,045)
- EU-H2020-ERC-ADV-2015 ERC Advanced grant van Blitterswijk ORCHESTRATE; Building complex life through self-organization: from organ to organism (2016-2020); € 2,655,000 (€ 2,655,000)
- Longfonds (Lung Foundation) Consortium Grant; Microengineered 3D analogues of alveolar tissue for lung regeneration (2015-2019); € 600,000 (€ 166,700)

Selected publications

- **Baptista, D., Teixeira, L., van Blitterswijk, C., Giselbrecht, S., Truckenmüller, R.** (2019). Overlooked? Underestimated? Effects of Substrate Curvature on Cell Behavior. Trends in Biotechnology, 37(8), 838-854. https://doi.org/10.1016/j.tibtech.2019.01.006
- Carvalho, M. R., **Barata, D., Teixeira, L. M., Giselbrecht, S.,** Reis, R. L., Oliveira, J. M., **Truckenmüller, R., Habibovic, P.** (2019). Colorectal tumor-on-a-chip system: A 3D tool for precision onco-nanomedicine. Science advances, 5(5), [1317]. https://doi.org/10.1126/sciadv.aaw1317
- **Barata, D.,** Dias, P., **Wieringa, P., van Blitterswijk, C., Habibovic, P.** (2017). Cell-instructive high-resolution micropatterned polylactic acid surfaces. Biofabrication, 9(3), [035004]. https://doi.org/10.1088/1758-5090/aa7d24
- **Beijer, N., Vasilevich, A.,** Pilavci, B., **Truckenmüller, R. K.,** Zhao, Y., Singh, S., Papenburg, B. J., **de Boer, J.** (2017). TopoWellPlate: A Well Plate Based Screening Platform to Study Cell–Surface Topography Interactions. Advanced Biosystems, 1(4), [1700002]. https://doi.org/10.1002/adbi.201700002
- **Vrij, E.,** Rouwkema, J., **LaPointe, V., van Blitterswijk, C., Truckenmüller, R., Rivron, N.** (2016). Directed Assembly and Development of Material-Free Tissues with Complex Architectures. Advanced Materials, 28(21), 4032-4039. https://doi.org/10.1002/adma.201505723
- Leferink, A., Schipper, D., Arts, E., **Vrij, E., Rivron, N.,** Karperien, M., Mittmann, K., **van Blitterswijk, C., Moroni, L., Truckenmüller, R.** (2014). Engineered Micro-Objects as Scaffolding Elements in Cellular Building Blocks for Bottom-Up Tissue Engineering Approaches. Advanced Materials, 26(16), 2592-2599. https://doi.org/10.1002/adma.201304539

Selected outreach activities

- "Gezond ouder worden met regeneratieve geneeskunde" ("RM to grow older in a healthy way"), *Changemakers Festival 2019*, Cube Design Museum, Kerkrade, October 2019. (https://changemakers-festival.eu/)
- "Sleutelen aan de mens" ("Fixing the human being") lecture organised by *New Scientist*, Amsterdam, December 2016. (https://www.facebook.com/events/pakhuis-de-zwijger/new-scientist-live-sleutelen-aan-de-mens/1792703117608778/)

Case study 3:

Osteoinductive biomaterials for bone regeneration

Background

Bone is one of the unique tissues in the body that has an excellent capacity to self-regenerate. A child with a broken arm only needs fixation by a cast for a few weeks to have the fracture heal scarlessly. Nevertheless, this natural regenerative potential fails when the defect is large or complex, also known as clinically relevant. As a result, loss and damage of bone tissue caused by trauma or disease, and lack of bone tissue as a result of congenital disorders, represent a clinical problem for over 20 million people annually. The standard and currently most effective treatment for bone defects consists of transplantation of autologous, i.e. a patient's own bone from elsewhere in the body to the defect area. This treatment is, however, associated with severe drawbacks. The patient needs to undergo a surgery at two different sites. Moreover, up to 40% of the patients experience pain as long as 10 years after bone graft was harvested. Most importantly, the amount of healthy bone that can be harvested is limited, and often insufficient for treating multiple defects. This problem of limited availability is becoming increasingly important with continuous ageing of the population and increased risk of new bone defects. Therefore, the need for substitutes for a patient's own bone is evident.

MERLN researchers have an established track record in translational research into bone graft substitutes. While various possibilities exist to develop alternatives for a patient's own bone, including the use of growth factors and other biologics, tissue engineered constructs based on a carrier material and a patient's own cells, MERLN researchers have predominantly made impact with their work on synthetic bone graft substitutes with the ability to induce new bone formation, the so-called osteoinductive biomaterials. Over the past 15 years, Prof. Pamela Habibovic and Prof. Clemens van Blitterswijk have contributed to the work that has led to development of calcium phosphate ceramics with a unique combination of chemical and structural properties that are as successful in healing critical-size bone defects as autologous bone is. These ceramics are fully synthetic, and can be produced in large quantities against relatively low cost, which is important to address the challenge of limited availability and to develop affordable treatments. Based on this work, a spin-off company, Progentix, was established in the early 2000's. This company has brought a product to the market and sold the license to NuVasive, one of the largest spine companies in the US. With this product, over 40000 patients requiring spinal fusion have been treated worldwide. Progentix is now a part of Kuros Biosciences and still develops new orthopedic products with osteoinductive capacity.

MERLN's approach

While the initial work on the topic of osteoinduction has led to clinical successes, there is still room for improvement regarding osteoinductive biomaterials. First, porous osteoinductive calcium phosphate ceramics are intrinsically brittle materials, which means that they cannot be used in load-bearing applications. Moreover, the challenge is to adapt these materials for patients with (chronic) diseases, such as osteoporosis or for defects resulting from removal of a tumour. Finally, the process of bone regeneration induced by osteoinductive materials should be further accelerated, while retaining their synthetic character. All these are current research topics within MERLN. The basis for this research is better understanding of fundamentals of the phenomenon of osteoinduction, with the rationale that understanding the contribution of individual properties on the bone regenerative potential of a material will aid design and development on new materials with improved performance.

Example: understanding the phenomenon of osteoinduction using proteomics. In a PhD project by Dr Ziryan Othman, in the group of Prof. Pamela Habibovic funded by an NWO TA Coast project "BioSurf", a family of calcium phosphates with known osteoinductive potential was used, in combination with advanced proteomics, to obtain deeper insights in the phenomenon of osteoinduction. This collaborative project with Erasmus University Rotterdam and University of Groningen has led to identification of protein profiles of human mesenchymal stromal cells cultured on osteoinductive and non-osteoinductive materials. Based on this work, plasma cell glycoprotein 1, encoded by the ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene was shown to play a role in osteoinduction by calcium phosphates. Importantly, this study also showed that the process of osteoinduction is triggered at the interface between the material and its surrounding, possibly triggered by the ion exchange dynamics occurring at the interface.

Example: Improving mechanical properties while retaining the osteoinductive potential. In the scope of the NWO Gravitation project "Materials-Driven Regeneration", Dr Yonggang Zhang, a talented post-doc in the group of Prof. Pamela Habibovic focuses on bottom-up building of ceramic and composite bone graft substitutes with unique mechanical properties. For example, he has developed a highly porous material consisting of two types on highly anisotropic nanosized calcium phosphates. Because of the unique structure of these calcium phosphates, and the way they are intertwined, the resulting material is deformable and possesses water-triggered shape-memory behaviour, which is special for a ceramic material. Similarly, in another project by Dr Zhang a composite material was developed consisting of unidirectionally oriented calcium phosphate crystals inside a polymer, with compressive strength similar to that of cancellous bone.

Example: Using bioinorganics to enhance osteoinductive potential. Bioinorganics are simple salts of, for example, zinc, strontium, or copper which are present in our body, often in trace amounts, and responsible for normal functioning of many organs and tissues. Also bone tissue comprises a number of bioinorganics, which have been shown to regulate various processes related to bone metabolism. To investigate whether these simple compounds can be used as an alternative to much more complex and expensive growth factors in enhancing the osteoinductive potential, the group of Prof. Pamela Habibovic has run several projects, including the one funded by her NWO Veni and Aspasia grants. Currently, within her NWO Vidi project "Bone Microfactory" a team is working on setting up a microfluidics-based platform for high-throughput production and screening of calcium phosphate biomaterials functionalized with (combinations of) bioinorganics, to identify potentially interesting candidates for effective and affordable bone graft substitutes.

Key points

- We are recognized worldwide for our work on synthetic biomaterials with osteoinductive potential.
- The work on osteoinduction continues both on the fundamental side, with the aim of unravelling the phenomenon of osteoinduction and on the translational side, focusing on improving mechanical and handling properties of osteoinductive biomaterials and making them suitable for more challenging clinical situations.

Collaborations

Internal:

- Prof. Peter Kessler (MUMC+, Craniomaxillofacial surgery) – on development of hybrid implants for cranial bone defects
- Prof. Ron Heeren (UM, M4I) – on mass spectrometry imaging of cell-biomaterial interactions

National:

- Dr Theo Luider (Erasmus Medica Center Rotterdam) and Reiner Bischoff (University of Groningen) – on proteomics
- Prof. Carlijn Bouten (Eindhoven University of Technology) – on correlation between vascular calcification and osteoinduction

International:
- Dr Marc Bohner (RMS Foundation) – on osteoinductive calcium phosphates
- Prof. Jake Barralet (McGill University) – on bioinorganics

Industry:
- Kuros Biosciences and Access2Bone – on synthetic bone graft substitutes
- Mimetas – on microfluidic platforms

Main grants
Total amount (MERLN part)
- NWO TA Coast "BioSurf" project (2012-2017); € 990,000 (€ 95,753)
- NWO Aspasia Habibovic "Combinorganics" project (2013-2018); € 200,000 (€ 150,000)
- NWO Vidi Habibovic "Bone Microfactory" project (2018-2022); € 800,000 (€ 800,000)
- NWO Gravitation "Materials-Driven Regeneration" program (2017-2027); € 25,565,000 (€ 8,521,000)
- NWO-TTW OTP "Craniomaxillofacial implants" project (2018-2022); € 749,382 (€ 494,822)

Selected publications

- Danoux, C. B. S. S., Bassett, D. C., **Othman, Z.,** Rodrigues, A. I., Reis, R. L., Barralet, J. E., **van Blitterswijk, C. A., Habibovic, P.** (2015). Elucidating the individual effects of calcium and phosphate ions on hMSCs by using composite materials. Acta Biomaterialia, 17, 1-15. https://doi.org/10.1016/j.actbio.2015.02.003
- Habraken, W., **Habibovic, P.,** Epple, M., Bohner, M. (2016). Calcium phosphates in biomedical applications: materials for the future? Materials Today, 19(2), 69-87. https://doi.org/10.1016/j.mattod.2015.10.008
- **Birgani, Z. T., Fennema, E.,** Gijbels, M. J., **de Boer, J., van Blitterswijk, C. A., Habibovic, P.** (2016). Stimulatory effect of cobalt ions incorporated into calcium phosphate coatings on neovascularization in an *in vivo* intramuscular model in goats. Acta Biomaterialia, 36, 267-276. https://doi.org/10.1016/j.actbio.2016.03.031
- Danoux, C., Sun, L., Kocer, G., **Birgani, Z. T., Barata, D.,** Barralet, J., **van Blitterswijk, C., Truckenmüller, R., Habibovic, P.** (2016). Development of Highly Functional Biomaterials by Decoupling and Recombining Material Properties. Advanced Materials, 28(9), 1803-1808. https://doi.org/10.1002/adma.201504589
- **Othman, Z.,** Fernandes, H., Groot, A. J., Luider, T. M., Alcinesio, A., **Pereira, D. D. M., Guttenplan, A. P. M., Yuan, H., Habibovic, P.** (2019). The role of ENPP1/PC-1 in osteoinduction by calcium phosphate ceramics. Biomaterials, 210, 12-24. https://doi.org/10.1016/j.biomaterials.2019.04.021

Selected outreach activities
- "Sleutelen aan de mens" ("Fixing the human being") lecture organised by *New Scientist*, Amsterdam, December 2016. (https://www.facebook.com/events/pakhuis-de-zwijger/new-scientist-live-sleutelen-aan-de-mens/1792703117608778/)
- "Regeneratieve geneeskunde" ("Regenerative Medicine") lecture, Gala van de Wetenschap (The Science Gala), Amsterdam, November 2017. (https://www.newscientist.nl/nieuws/het-gala-van-de-wetenschap-2017-biedt-hoop-in-donkere-tijden/)
- Interview for WijLimburg TV 1op1 (https://www.wijlimburg.nl/nieuws-overzicht/wijlimburg-tv/1op1-pamela-habibovic-brightlands-maastricht-health-campus/), February 2018.
- "Regenerative medicine: a biological boost", invited lecture at the Royal Institution, London, October 2018. (http://www.rigb.org/whats-on/events-2018/october/public-regenerative-medicine-a-biological-boost)
  Lecture: https://www.youtube.com/watch?v=Mu9YkBY3IQY
  Q&A: https://www.youtube.com/watch?v=KrFJsfHxRjM
- "Eyeopener – slimme korreltjes voor betere botten / smart particles for better bones", February 2021. (https://nbv.kncv.nl/k/nl/n1099/news/view/176609/104679/eye-opener-van-de-week-pamela-habibovic.html)

Case study 4:

Nanotechnology for stem cell tracing and gene delivery

Background
Nanotechnology for stem cell tracing
Stem cells have great therapeutic potential due to their inherent ability to differentiate into different cell types and their capacity for self-renewal. As such, stem cells offer potential new treatment options for many prevalent chronic and degenerative disorders. However, only a few stem cell treatments have been approved for clinical use after safety and efficacy tests. The lack of knowledge of *in vivo* stem cell fate after transplantation represents a significant bottleneck in their clinical translation. New tools are needed to improve our understanding of stem cell fate to enable optimization of stem cell processing and transplantation strategies, in turn enabling faster clinical translation of stem cell therapies with a higher success rate.

Gene delivery
RM promises to regrow tissues and organs such that the repaired unit will be indistinguishable from its native counterpart. A key component of this process involves guiding the behaviour of the cells that will generate the new tissues. Under physiological conditions, cell behaviour is regulated by numerous signals, including proteins. It follows that several approaches have been investigated to achieve successful protein delivery. Yet, the clinical efficacy has been disappointing and adverse side effects have been reported. As an alternative, local gene delivery is emerging as a promising approach. The idea is that nucleic acids can be delivered intracellularly to result in the in situ production of proteins. Significant preclinical progress has been made, but the disadvantages of current genetic delivery strategies are the cost, safety concerns, and the regulatory complexity.

MERLN's approach
At MERLN, the group of Dr Sabine van Rijt develops imaging probes based on nanoparticles that enable stem cell tracing. In this work, we combine nanomaterials with inherent optical properties in a single construct to allow imaging across multiple techniques. Using these probes, we work together with several partners to optimize stem cell therapies. For example, in a collaborative effort with the University Medical Center Utrecht, we traced mesenchymal stem cell migration to the brain in a mouse model of encephalopathy of prematurity, a common cause of long term neurodevelopmental morbidity in extreme preterm infants. We showed that this migration was significantly increased when there was inflammation in the brain. These findings improved our understanding of the efficacy and mode of action of this new stem cell therapy, aiding its clinical translation. We also work together with several other partners within UM and internationally within large EU consortia to trace stem cells *in vivo* and to combine stem cell tracing with functional imaging.

Dr Elizabeth Rosado Balmayor's group investigates the use of protein-coding DNA and RNA for tissue regeneration purposes. We combine DNA with viral (e.g. adenovirus) or non-viral vectors (e.g. liposomes or dendrimers) to form particulate complexes able to internalize their genetic cargo inside the cell. We combine these complexes with biomaterials to develop gene activated matrices. We were able to show that mesenchymal stem cells transduced with an adenovirus carrying the human bone morphogenetic protein 2 (BMP-2) cDNA are able to enhance neo-bone formation *in vivo* using a rat critical-sized femur defect. This study showed high efficacy and safety. An ex-vivo gene therapy approach was followed avoiding in-vivo administration of the virus. Nevertheless, concerns of safety and cost-efficacy remain when using viral gene therapy. We have pioneered the development of a new, chemically modified mRNA (cmRNA) for bone regeneration. By introducing a series of chemical modifications to the mRNA structure, we have eradicated issues of stability, toxicity, and immunogenicity of the mRNA. As part of our investigations, we developed a BMP-2 cmRNA that showed robust BMP-2 production *in vitro* (stem cells) resulting in upregulation of osteogenic and angiogenic genes. We conducted investigations in a rat critical-sized femoral defect, where BMP-2 cmRNA induced complete bridging of the bone defect. At present, as part of an NIH-funded study, we are investigating the efficacy and

safety of this technology in a large, orthopedically relevant animal model. On the other hand, we hypothesize that several cmRNAs could be administered to simultaneously stimulate relevant tissue regeneration processes, (i.e. osteogenesis, angiogenesis, and innervation). Together with Prof. Martijn van Griensven we are investigating the application of a cocktail of cmRNAs *in vitro* using stem cells and *in vivo* in relevant models. These investigations are part of the EU H2020 funded project cmRNAbone.

Key points

- Our nanotechnology is used in collaboration with other groups to improve our understanding of stem cell fate, enabling optimization of stem cell processing and transplantation strategies.
- We are pioneers in the use of cmRNA for musculoskeletal tissue regeneration.

Collaborations

Internal:

- Prof. Bert Smeets (UM, GROW - Toxicogenomics) – on developing nano-probes that can trace and repair mesangioblasts to treat muscle disease
- Prof. Tim Wolfs (UM, GROW – Pediatrics) – on developing nanoparticles for stem cell tracing in large animal models to improve cell based therapies for preterm babies
- Prof. Han Brunner and Dr Masoud Zamani Esteki (MUMC+, Department of Clinical enetics) – on using barcoded nanoparticles for lineage tracing in IVF embryos

National:

- Prof. Cora Nijboer (University Medical Center Utrecht) – on using multimodal nanoparticles to trace stem cells in mouse model of premature babies

International:

- Prof. Christopher H. Evans (Mayo Clinic) – on gene therapies for musculoskeletal tissue healing with particular emphasis on cmRNA for bone healing
- Prof. Andrea Banfi (University of Basel, Switzerland) – on cmRNA for angiogenesis
- Prof. Martin Stoddart (AO Faoundation, Switzerland) - on materials for cmRNA delivery
- Prof. Fergal O'Brien (RCSI, Ireland) – on biomaterials for microRNA delivery
- Prof. Lizette Morejon and Prof. Jose Angel Delgado (University of Havana, Cuba) – on bioglasses of natural origin for gene incorporation

Industry:

- Ethris GmbH – on cmRNA construct design and production
- OzBioscience – on lipids for gene delivery
- Kuros Biociences – on biomaterials for gene delivery
- PreSens – on O_2 sensors to track cellular metabolic activity upon transfection

Main grants

Total amount (MERLN part)

- EU-H2020-SC1-BHC-07-2019 "PREMSTEM" project (2020-2025); € 8,999,620 (€ 207,313)
- EU-H2020-SC1-BHC-07-2019 "JOINTPROMISE" project (2020-2024); € 7,901,115 (€ 1,694,275)
- ZonMw TOP Vision (2018-2023) project; € 675,000 (€ 412,437)
- EU-H2020-SC1-BHC-07-2019 "cmRNAbone" project (2019-2024); € 6,256,759 (€ 999,749)
- NIH R01AR074395 "Use of Chemically Modified RNA to Enhance Bone Healing" project (2019-2024); $ 2,536,712 ($ 481,535)

Selected publications

- Vaes, J. E. G., van Kammen, C. M., **Trayford, C.,** van der Toorn, A., Ruhwedel, T., Benders, M. J. N. L., Dijkhuizen, R. M., Möbius, W., **van Rijt, S. H.,** Nijboer, C. H. (2021). Intranasal mesenchymal stem cell therapy to boost myelination after encephalopathy of prematurity. Glia, 69(3), 655-680. https://doi.org/10.1002/glia.23919
- **Rosenbrand, R., Barata, D., Sutthavas, P.,** Mohren, R., Cillero-Pastor, B., **Habibovic, P., van Rijt, S.** (2018). Lipid surface modifications increase mesoporous silica nanoparticle labeling properties in mesenchymal stem cells. International Journal of Nanomedicine, 13, 7711-7725. https://doi.org/10.2147/IJN.S182428
- Fayed, O., **van Griensven, M., Birgani, Z. T.,** Plank, C., **Balmayor, E. R.** (2021). Transcript-activated coatings on titanium mediate cellular osteogenesis for enhanced osteointegration. Molecular Pharmaceutics, 18(3), 1121-1137. https://doi.org/10.1021/acs.molpharmaceut.0c01042
- Zhang, W., **De La Vega, R. E.,** Coenen, M. J., Müller, S. A., **Peniche Silva, C. J.,** Aneja, M. K., Plank, C., **van Griensven, M.,** Evans, C. H., **Balmayor, E. R.** (2019). An Improved, Chemically Modified RNA Encoding BMP-2 Enhances Osteogenesis *in vitro* and *in vivo*. Tissue Engineering. Part A , 25(1-2), 131-144. https://doi.org/10.1089/ten.TEA.2018.0112
- Müller, C. W., Hildebrandt, K., Gerich, T., Krettek, C., **van Griensven, M., Rosado Balmayor, E.** (2017). BMP-2-transduced human bone marrow stem cells enhance neo-bone formation in a rat critical-sized femur defect. Journal of Tissue Engineering and Regenerative Medicine, 11(4), 1122-1131. https://doi.org/10.1002/term.2015
- **De la Vega, R. E.,** Atasoy-Zeybek, A., Panos, J., **van Griensven,** M., Evans, C. H., **Balmayor, E. R.** (2021). Gene Therapy for Bone Healing: Lessons Learned and New Approaches. Translational Research, In Press. https://doi.org/10.1016/j.trsl.2021.04.009

Case study 5:

In silico tools for RM

Background

Understanding the complex interactions of cells with biomaterials plays an increasingly pivotal role in the development of novel biomedical devices and tissue engineered constructs. Considering the huge amount of material parameter combinations that need to be investigated to identify the optimal cell responses, advanced high-throughput screening platforms have been developed, allowing to explore the complex interactions of cells with particular biomaterial properties (e.g. surface chemistry, surface topography or combinations thereof) in an efficient and controlled manner. However, the amount of data created by high-throughput screening methods is overwhelming, requiring advanced computational methods to find patterns in cellular responses and make statistical predictions on cell-material interactions.

MERLN's approach

At MERLN, the group of Dr Aurélie Carlier focuses on developing *in silico* models of cell-biomaterial interactions. For example, in a collaborative effort with Nottingham University, we have investigated the influence of material topography on macrophage attachment and phenotype as macrophages play a central role in orchestrating immune responses to foreign materials. We analyzed the high-throughput screening data of human monocyte–derived macrophages on a library of 2176 different micropatterns and built a model that correlates cell attachment and phenotype with a selection of surface descriptors. Similar *in silico* tools were developed and used to discover surface topographies that control ICAM-1 expression, modulate the pluripotency in human induced pluripotent stem cells, determine the cytokine secretion profile and influence the tenogenic phenotype. Moreover, we have developed advanced tools to design optimal novel surface topographies *in silico*.

In order to further build on the potential of advanced *in silico* tools, we have created a publicly accessible repository called The Compendium for Biomaterial Transcriptomics (cBiT). cBiT is a data warehouse that gives users the opportunity to search through biomaterial-based transcriptomics data sets using a web interface. Data of interest can be selected and downloaded, together with associated measurements of material properties. We aim to make cBiT the hub for biomaterial-associated data, thereby enabling major contributions to a more efficient development of new materials.

The group is recently extending this work towards white box mechanistic models of cell-biomaterial interactions, based on the most important components identified through the above-mentioned machine learning methods. Such computational simulations can test hypotheses and perform experiments *in silico,* many orders of magnitude faster than real experiments. Moreover, these *in silico* models also allow making extrapolations to patterns that are not present in the original data, overcoming some limitations of the data-driven models.

Key points
- We have demonstrated that *in silico* tools can be used to identify optimal surface topographies for inducing particular cell behaviour.
- We have shown that cellular responses such as bacterial attachment, stem cell attachment and differentiation, can be predicted from mathematical biomaterial descriptors.
- We have created advanced methods showing that computational models of material-cell interactions can screen a larger materials space by producing a model from known interactions and applying the model to a virtual library of materials.

Collaborations
Internal:
- Prof. Katerina Stankova (UM, Department of Knowledge Engineering) – on computational modelling of dynamical systems
- Dr Rachel Cavill (UM, Department of Knowledge Engineering) – on computational modelling of dynamical systems)
- Prof. Jos Prickaerts (UM, MHeNs) – on computational modelling of cAMP signalling
- Prof. Michel Dumontier (UM, Institute of Data Science) – on databases and ontologies for biomaterials

National:
- Prof. Jan de Boer (Eindhoven University of Technology) – on cell-biomaterial interactions
- Prof. Patricia Dankers (Eindhoven University of Technology) – on synthetic biomaterials to control integrin signaling
- Prof. Roos Masereeuw (University of Utrecht) – on modeling toxin transport and kidney organoid development for a bioartificial kidney

International:
- Prof. Morgan Alexander (Nottingham University, United Kingdom) – on surface topography screening for bacterial attachment
- Prof. Viola Vogel (ETH Zurich, Switzerland) – on integrin signaling during fibroblast to myofibroblast transition and wound regeneration
- Prof. Leslie Loew and Prof. Ann Cowan (University of Connecticut, USA) – on developing advanced simulation tools
- Prof. Haguy Wolfenson (Technion University, Israel) – on modelling integrin adhesome signalling
- Prof. Liesbet Geris (KULeuven, Belgium) – on computational modelling of bone regeneration

Main grants
Total amount (MERLN part)
- NWO Veni Carlier project; "Yoga for bone cells: *in silico* modelling of cell shape transduction to advance osseointegrative implant design" (2016-2021); € 250,000 (€ 250,000)
- EU-H2020-MSCA-ITN-2015 ETN project "Tendon Therapy Train" (2016-2021); € 3,876,966 (€ 510,749)

Selected publications

- Vassey, M. J., Figueredo, G. P., Scurr, D. J., **Vasilevich, A. S., Vermeulen, S., Carlier, A.,** Luckett, J., **Beijer, N. R. M.,** Williams, P., Winkler, D. A., **de Boer, J.,** Ghaemmaghami, A. M., Alexander, M. R. (2020). Immune Modulation by Design: Using Topography to Control Human Monocyte Attachment and Macrophage Differentiation. Advanced Science, 7(11), [1903392]. https://doi.org/10.1002/advs.201903392
- Leuning, D. G., **Beijer, N. R. M.,** du Fosse, N. A., **Vermeulen, S.,** Lievers, E., van Kooten, C., Rabelink, T. J., **de Boer, J.** (2018). The cytokine secretion profile of mesenchymal stromal cells is determined by surface structure of the microenvironment. Scientific Reports, 8, [7716]. https://doi.org/10.1038/s41598-018-25700-5
- **Vasilevich, A., Carlier, A.,** Winkler, D. A., Singh, S., **de Boer, J.** (2020). Evolutionary design of optimal surface topographies for biomaterials. Scientific Reports, 10(1), [22160]. https://doi.org/10.1038/s41598-020-78777-2
- **Vasilevich, A. S., Carlier, A., de Boer, J.,** Singh, S. (2017). How Not To Drown in Data: A Guide for Biomaterial Engineers. Trends in Biotechnology, 35(8), 743-755. https://doi.org/10.1016/j.tibtech.2017.05.007
- **Hebels, D. G. A. J., Carlier, A.,** Coonen, M. L. J., Theunissen, D. H., **de Boer, J.** (2017). cBiT: A transcriptomics database for innovative biomaterial engineering. Biomaterials, 149, 88-97. https://doi.org/10.1016/j.biomaterials.2017.10.008
- **Karagöz, Z.,** Rijns, L., Dankers, P. Y. W., **van Griensven, M., Carlier, A.** (2021). Towards understanding the messengers of extracellular space: Computational models of outside-in integrin reaction networks. Computational and Structural Biotechnology Journal, 19, 303-314. https://doi.org/10.1016/j.csbj.2020.12.025

Case study 6:

Orthopaedics and craniomaxillofacial surgery

Background
The increase in life expectancy and the aging of the population in developed countries have led to an escalation in the incidence of musculoskeletal diseases worldwide. According to a World Health Organization report, "this increase will continue, especially in developing countries due to the pernicious effects of urban development and motorization." (WHO report 2009. http://www.who.int/ncd /cra). By 2050, almost 40 percent of Europeans will be over the age of 60.

Musculoskeletal diseases have a huge socioeconomic impact, of a magnitude equal to or greater than cardiovascular diseases or cancer and it is estimated that their total cost represents around 2.5% of the GDP of developed countries. Today, 80% of European people over age of 65 has at least one chronic disease. One in six have a mild or severe disability and 10% of population have to leave jobs due to health problems. €240 billion are lost in EU productivity due to absence and 80% of the EU healthcare budget of €200 billion is spent on chronic diseases. Healthy aging does not mean absence of disease but a good control of it, to allow a fully functional life, with total mobility, socializing and contribution to society. For this, high-quality care is needed.

Injuries of the musculoskeletal system, including bone, cartilage, tendons, ligaments, joints, etc. are common and their incidence increases with ageing. While there have been important advances in research and development of therapies for treatment of musculoskeletal tissues in the last two decades, their translation to clinical care and the industrial

development are still not effective. This is in a part due to the fact that musculoskeletal pathologies present different symptoms, etiologies, co-morbidities (e.g. osteoporosis, diabetes) and affect patient of different ages and at different sites. Therefore, there is a need for patient-specific therapies, that depend not only on the type of injury but also on the patient condition. To address this need, it is important to combine (clinical) biology, engineering and biomaterials science.

MERLN's approach

MERLN's researchers have a strong track record in musculoskeletal regeneration and many PIs have active research lines in this field, including Prof. Martijn van Griensven, Prof. Pamela Habibovic, Prof. Lorenzo Moroni, Dr Rosado Balmayor, and others. We use biomaterials, cells, biologics and combinations thereof to develop effective treatments for bone, cartilage, osteochondral and tendon and ligament injuries. For example, for bone regeneration, we have developed ceramic materials with osteoinductive properties (see also case study 3), but we also work on bioglasses, polymeric materials and hybrid materials combining two or more of these material types. A common cell source are mesenchymal stromal cells, derived from bone marrow or adipose tissue, and in collaboration with the trauma surgery of MUMC+, we investigate different methods to efficiently isolate cells. To further enhance the bone regenerative potential, we also use cmRNA (see Case study 4), miRNA as well as bioinorganic ions. For cartilage, tendon and osteochondral regeneration, biofabrication strategies (Case study 1) are commonly used, while for regenerating the bone-to-tendon interface, microfluidic techniques are used (Case study 2).

To support the development of musculoskeletal regenerative therapies, we do research into the effect of fracture hematoma, immune system as well as ageing on the regenerative potential of a patient. Moreover, *in silico* models of bone fracture healing and various regenerative processes are developed.

Key points

- We have an excellent track record in musculoskeletal regeneration for orthopedics and craniomaxillofacial surgery, ranging from fundamental to highly translational research.
- Musculoskeletal regeneration is one of the best examples of MERLN's research where materials science, biology, technology and medical sciences are combined and synergized.

Collaborations

Internal:

- Dr Pieter Emans and Dr Chris Arts (MUMC+, Orthopedics) – on osteochondral regeneration and bioglasses, respectively

- Prof. Peter Kessler (MUMC+, Craniomaxillofacial Surgery) – on hybrid implants for cranial defect
- Prof. Martijn Poeze and Dr Taco Blokhuis (MUMC+, Trauma) – on fracture healing and non-unions

National:

- Prof. Sander Leeuwenburgh (Radboud University Medical Center) – on inorganic and nanomaterials
- Prof. Moyo Kruyt (University Medical Center Utrecht) – on osteoinductive materials in spinal fusion

International:

- Prof. Rui Reis, Dr Isabel Leonor (University of Minho, Portugal) – on osteochondral regeneration
- Prof. Markus Huber-Lang (Ulm University, Germany) and Prof. Frank Hildebrand (RWTH Aachen, Germany) – on porcine polytrauma model for fracture healing and the immune system studies
- Prof. Martin Stoddart (AO Foundation, Switzerland) and Prof. Andrea Banfi (University of Basel, Switzerland) – on cmRNA for osteoporotic fractures
- Prof. Maria-Rosa Alguilar de las Armas and Prof. Julio San Roman, Dr Blanca Vazquez (CSIC, Spain), Prof. Fergal O'Brien (Royal College of Surgeons, Ireland) – on miRNA for osteoporosis and osteogenesis imperfecta treatment
- Prof. Christopher H. Evans (Mayo Clinic, USA) – on cmRNA for large bone defects
- Dr Riccardo Gottardi (University of Pennsylvania) – on enthesis research
- Dr Alejandro Gurostovich (Ucasal, Argentina) – on zebrafish model for vascularization
- Prof. Lizette Morejón, Prof. José A. Delgado (University of Havanna, Cuba) – on bioglass and osteochondral scaffolds

Industry:

- Kuros Biosciences – on osteoinductive biomaterials
- Presens – on implantable, real-time oxygen sensors for monitoring regeneration
- Osteopore – on GMP 3D printing for clinical applications
- Ethris – on cmRNA for bone regeneration

Main grants

Total amount (MERLN part)

- EU-H2020-SC1-BHC-07-2019 "JOINTPROMISE" project (2020-2024); € 7,901,115 (€ 1,694,275)
- EU-H2020-SC1-BHC-07-2019 "cmRNAbone" project; € 6,257,759 (€ 999,749)
- NIH R01AR074395 "Use of Chemically Modified RNA to Enhance Bone Healing" project (2019-2024); $ 2,536,712 ($ 481,535)
- MDR Young Talent Grant "Fishing for growth factors" project (2020-2021): € 10,000 (€ 10,000)
- CSC "Bottom-up bone tissue engineering" project and "Poly (Ester Amide) Reinforced Hydrogels" project (2020-2024); 2x € 64,800 (2x € 64,800)
- ON-Foundation Starter Grant "Tendon" project (2020-2021); € 9,265 (€ 9,265)
- NWO-ENW Incentive Grant for Women in STEM Grant "BIOTETRIS" project (2021-2022); € 147,100 (€ 147,100)
- Mudanjiang First People's Hospital "Fracture hematoma" project (2020-2024); € 86,400 (€ 86,400)

Selected publications

- **Othman, Z.,** Mohren, R. J. C., Cillero-Pastor, B., Shen, Z., Yves S.N.W. Lacroix, Y. S. N. W., **Guttenplan, A. P. M., Birgani, Z. T.,** Eijssen, L., Luider, T. M., **van Rijt, S., Habibovic, P.** (2020). Comparative proteomic analysis of human mesenchymal stromal cell behaviour on calcium phosphate ceramics with different osteoinductive potential. Materials Today Bio, 7, [100066]. https://doi.org/10.1016/j.mtbio.2020.100066
- Payr, S., Schuseil, T., Unger, M., Seeliger, C., Tiefenbroeck, T., **Balmayor, E. R., van Griensven, M.** (2020). Effect of donor age and 3D-cultivation on osteogenic differentiation capacity of adiposederived mesenchymal stem cells. Scientific Reports, 10, [10408]. https://doi.org/10.1038/s41598-020-67254-5
- **Camarero-Espinosa, S., Tomasina, C., Calore, A., Moroni, L.** (2020). Additive manufactured, highly resilient, elastic, and biodegradable poly(ester)urethane scaffolds with chondroinductive properties for cartilage tissue engineering. Materials Today Bio, 6, [100051]. https://doi.org/10.1016/j.mtbio.2020.100051
- **Koper, D.,** ter Laak-Poort, M., Lethaus, B., Yamauchi, K., **Moroni, L., Habibovic, P.,** Kessler, P. (2019). Cranioplasty with patient-specific implants in repeatedly reconstructed cases. Journal of Cranio-Maxillofacial Surgery, 47(5), 709-714. https://doi.org/10.1016/j.jcms.2019.01.034
- **Moroni, L.,** Nandakumar, A., Barrere-de Groot, F., **van Blitterswijk, C. A., Habibovic, P.** (2015). Plug and play: combining materials and technologies to improve bone regenerative strategies. Journal of Tissue Engineering and Regenerative Medicine, 9(7), 745-759. https://doi.org/10.1002/term.1762

Selected outreach activities

- "Stel je voor: een verloren been groeit vanzelf aan" ("Imagine, a lost bone regrows") Interview in "Trouw", 2018. (https://www.trouw.nl/nieuws/stel-je-voor-een-verloren-been-groeit-vanzelf-aan~b4d095f4/)
- Interview on RTL Nieuws (EditieNL) about the treatment of a patient with a long bone defect, December 2020. https://www.rtlnieuws.nl/tech/video/5205347/3d-printer-voorkomt-amputatie

Case study 7:

Corneal regeneration

Background

The cornea is the window through which light enters the eye. The World Health Organization estimates that over 5 million people are blind in both eyes from corneal disease (e.g. infections, degenerations and dystrophies). The societal burden is substantial, given the impact on employment, quality of life, and related caretaking requirements. While corneal transplantation (keratoplasty) is a treatment for some corneal diseases, it is not suitable for all indications, and the number of donor corneas cannot match the global demand. An estimated 25 million people worldwide are waiting for sight-saving procedures. For both the patient and the wider society, there is an urgent need to develop therapies to regenerate the cornea and prevent blindness. The cornea is composed of multiple cell layers, each with their distinct characteristics: the outer surface of stratified epithelial cells, the middle layer of stromal keratocytes, and the inner layer of tightly packed endothelial cells. Therapies can be developed for the cell layers in isolation, or as a complete corneal construct.

MERLN's approach

In collaboration with the Department of Ophthalmology at the MUMC+, the MERLN Institute is working on a variety of regenerative solutions for all layers of the cornea. At the center of these collaborations are the corneal surgeons Prof. Rudy Nuijts and Dr Mor Dickman. Since 2020, Dr Dickman has a joint appointment with the MERLN Institute to co-lead a research line with Dr Vanessa LaPointe.
Regenerating the cornea is challenging due to its demanding optical properties, presenting an opportunity for new technology and approaches. At MERLN, we are using micro/nanotechnology, materials science, (stem) cell biology, and gene editing technologies to regenerate the cornea. All MERLN departments and multiple PIs are involved in these research lines.

Example: Dealing with donor shortage in Fuchs' endothelial corneal dystrophy (FECD). FECD is marked by the progressive degeneration of the endothelial cells on the inner surface of the cornea. As these cells are lost, the cornea retains excess fluid, resulting in diminished vision. FECD affects about 4% of the population over 40 years old, with a strong female predominance. The only effective treatment is corneal transplantation, but there is a worldwide shortage of donor corneas. The InSciTe Chemelot–funded project entitled "Biomedical solutions for better EyeSciTe" is a ~€12 M research program led by MERLN comprising academic applicants from the UM, MUMC+, Eindhoven, University of Technology and the Weizmann Institute (Israel), as well as industrial partners from DSM, Euro Tissue Bank (ETB-Bislife), and SupraPolix B.V. With an approach based on (stem) cell biology and RM technology (especially materials science and microfabrication), the program has multiple aims related to developing artificial corneas and corneal lenses. To advance our therapies for diseases of the corneal endothelium, we sent a PhD candidate to the world leading group of Prof. Jodhbir Mehta at the Singapore National Eye Center to learn primary endothelial cell culture and isolation. Having brought this knowledge back to UM, we are now working on complementary strategies of a biomaterials-based carrier for cell transplantation and stem cell differentiation to generate a new cell source.

Example: Tracking stem cell transplantations for bilateral limbal stem cell deficiency (LSCD). Corneal epithelial cells undergo continuous renewal from the limbal stem cells residing at the periphery of the cornea. Minor injuries can be readily repaired, but if the limbus is damaged following severe injury or congenital deficiency, this repair cannot occur. The result is the growth of the opaque conjunctive over the cornea, resulting in intense pain and blindness in the most severe cases. Current treatment consists of a stem cell therapy wherein the patient receives a cultured graft of limbal stem cells to restore their vision. In our ZonMW-funded project entitled VISION: Uncovering stem cell–based corneal regeneration using nanotechnology and multimodal imaging, we aim to improve our understanding of this stem cell therapy. We developed nanoparticle probes to trace these cells and better understand what happens upon their transplantation, thereby informing and improving the success rates of the therapy. This €675K collaborative grant was awarded to Prof. Clemens van Blitterswijk (MERLN), Prof. Rudy Nuijts (MUMC+), and Dr Stefano Ferrari (Veneto Eye Bank, Italy), with Dr Sabine van Rijt as the Project Leader.

Key points

- We have established the capability of culturing primary human corneal endothelial cells, opening up the possibility of providing a RM therapy to patients.
- We have developed nanotechnology that can be used to trace limbal stem cells in real-time, which will improve our understanding on how stem cells regenerate the cornea.

Collaborations

Internal:

- Prof. Rudy Nuijts and Dr Mor Dickman (MUMC+, Ophthalmology) – on cornea regeneration

National:

- Prof. Patricia Dankers (Eindhoven University of Technology) – on biomaterials for corneal regeneration

International:

- Dr Stefano Ferrari (Veneto Eye Bank, Italy) – on limbal stem cells
- Prof. Jodhbir Mehta, Dr Gary Peh (Singapore National Eye Center, Singapore) – on corneal endothelial cells

Industry:

- DSM – on biomaterials for corneal regeneration
- ETB-Bislife – on tissue for research
- Single Cell Discoveries – on single cell RNA sequencing
- Suprapolix – on biomaterials for corneal regeneration

Main grants

Total amount (MERLN part)

- Chemelot InSciTe EyeSciTe (2016–2021); € 6,363,800 (€ 3,321,846)
- Fulbright U.S. Student Award - Kwasi Amofa (2017); travel costs (€ 0)
- Whitaker International Fellows and Scholars Award - Kwasi Amofa (2017); $ 10,000 ($ 10,000)
- ZonMw TOP Vision (2018-2023) project; € 675,000 (€ 412,437)
- Kootstra Talent Fellowship Programme Dickman (2017-2018); 1 year PD salary (1 year PD salary)
- UM MHeNS PhD call (2021–2025); 4 years PhD salary (4 years PhD salary)
- ZonMw Veni Dickman (2019-2022): € 250,000 (€ 0)
- ZonMw Enabling Technologies Hotel (2019): € 29,887 (€ 29,887)

Selected publications

- **Català, P., Vermeulen, W., Rademakers, T.,** van den Bogaerdt, A., Kruijt, P., Nuijts, R. M. M. A., **LaPointe, V. L. S., Dickman, M. M.** (2020). Transport and Preservation Comparison of Preloaded and Prestripped-Only DMEK Grafts. Cornea, 39(11), 1407-1414. https://doi.org/10.1097/ICO.0000000000002391
- **van Velthoven, A. J. H.,** Bertolin, M., Barbaro, V., **Sthijns, M. M. J. P. E.,** Nuijts, R. M. M. A., **LaPointe, V. L. S., Dickman,** M. M., Ferrari, S (2020). Increased cell survival of human primary conjunctival stem cells in dimethyl sulfoxide–based cryopreservation media. Biopreservation and Biobanking, 19(1), 67-72. https://doi.org/10.1089/bio.2020.0091
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Case study 8:

Type 1 diabetes

Background

Type 1 diabetes (T1D) is an autoimmune disease hallmarked by the specific destruction of the insulin producing beta cells in the so-called islets of Langerhans residing in the pancreas. Exogenous insulin therapy is the main method to prevent hyperglycemic events in T1D patients. Although very effective, the long-term effects of a disbalance in carbohydrate intake and insulin therapy leads ultimately to a number of complications, such as microvascular damage causing neuropathy, blindness and diabetic nephropathy, and macrovascular damage, leading to atherosclerosis and potential heart disease. Besides these physical effects, there is also a psychological factor involved, caused by the continuous stress and frustration due to long-term difficulties with blood glucose management, wearing the T1D patient and supporting family members down and reducing overall quality of life. Beta cell replacement therapy, a method in which the lost beta cells are replaced by a transplantation of donor islets of Langerhans, i.e., clinical islet transplantation (CIT) has been shown to be very effective in restoring normoglycemia. The downside of CIT is that this therapy needs to be accompanied by immunosuppressive therapy, to prevent immune rejection of the allogenic tissue, which significantly increases the risk of infection, certain types of cancers, and even has been shown to have some negative effects on beta cells, and alpha and beta cell regeneration. There is also a lack of donors to sufficiently supply all patients with islets, forcing the treating physician to balance risks versus benefits before a patient is deemed eligible for replacement therapy. The therapy itself is also not without risks. The biosynthesis and release of insulin from beta cells may be negatively affected by oxidative and endoplasmic reticulum stress, inflammation, allo- and autoimmunity, hypoxia and lack of nutrients. This multifactorial reaction causes 60% of the islets to die within the first week after CIT. These complicating factors need to be taken into account when developing islet delivery devices, in which islets are embedded or encapsulated prior to implantation.

MERLN's approach

In MERLN's "Islet research group" led by Dr Aart van Apeldoorn, we are working on the development of multiple beta cell delivery strategies. The most important one includes an open delivery device, in which beta cells are distributed in a controlled manner through a specific device geometry while a predesigned pore distribution allows for vascularisation of the embedded cells ensuring maximum survival and function. Another type of device which is currently being developed is an immunoprotective beta cell delivery device. In this device beta cells can be embedded while they are protected from the recipient's immune system. The latter is especially interesting since it can avoid the use of immunosuppressive therapy, while at the same time it can protect the recipient from any rogue undifferentiated stem cells which could form teratomas after transplantation.

These devices will prevent the highest peaks and troughs in blood sugar levels and will therefore reduce many serious additional consequences of diabetes. The burden of disease will decrease and the quality of life will increase, allowing patients to be more active in life. Both devices are at an advanced stage of development and the aim is to start a first phase clinical safety trial with the open device in the RegMed XB consortium.

Another device strategy which has just received funding is the development of a new beta cell delivery strategy without the use of synthetic biomaterials. This research will predominantly focus on the combination of living cells and natural biomaterials to create an endocrine insulin delivery organ suitable for transplantation. In this way, any adverse reaction against a synthetic biomaterial can be averted while the pancreatic islet niche can be mimicked in the best possible way, leading to a biological delivery system.

Key points

- We are doing highly focused translational research aiming to significantly improve existing beta cell replacement strategies benchmarked against clinical islet transplantation.
- Our research is strongly supported by health foundations including the DON (Diabetes Type I) and diabetes research foundation in the Netherlands and Juvenile Diabetes Research Foundation in the US, as well as by governmental funding supplied by Health Holland.

Collaborations

Internal:

- Prof. Ron Heeren (UM, M4I) – on mass spectrometry analysis of islet extracellular matrix
- Dr Tim Wolfs (MUMC+, Pediatrics) – on sheep islets and pancreas isolation for research purposes

National:

- Prof. Patricia Dankers (Eindhoven University of Technology) – on the development of new biomaterials for beta cell delivery devices
- Prof. Kitty Nijmeier and Dr Zandrie Borneman (Eindhoven University of Technology) – on methods for membrane analysis
- Prof. Niels Geijsen (Leiden University Medical Center) – on reporter cell lines for de novo beta cells
- Prof. Eelco de Koning (Leiden University medical center and Hubrecht Institute Utrecht) – on clinical islet transplantation and stem derived beta cells and diabetes animal models
- Dr Françoise Carlotti (Leiden University Medical Center) – on small animal studies for type 1 diabetes and de novo beta cell development
- Dr Arnaud Zaldumbide (Leiden University Medical Center) – on beta cell stress studies
- Dr Volkert Huurman (Leiden University Medical Center) – on clinical implementation and trials for beta cell replacement devices

International:
- Dr Albert Hwa and Prof. Gordon Weir (Joslin Diabetes Institute and Harvard Medical, Boston MA, USA) – on new beta cell transplantation techniques
- Prof. François Pattou (Lille University, INSERM, Lille, France) – on minipig models for islet transplantation and beta cell delivery devices
- Prof. Shane Grey (Garvan institute, Sydney, Australia) – on inflammation and islet transplantation
- Dr Tiago Henriques da Silva (University of Minho, Portugal) – on fucoidan to alleviate cell stress
- Prof. Pierre Gianello (Université Catholique de Louvain UCL, Brussels, Belgium) – on porcine islets
- Dr Hanne Scholz (Oslo University Hospital, Oslo, Norway) – on experimental islet transplantation and 3D beta cell replacement devices
- Dr Liesbet Geris (University of Liège, Belgium) – on mathematical modelling of oxygen in beta cell delivery devices

Industry:
- VELDLASER - on laser micromachining of biomaterial polymer films for cell delivery devices
- Fujifilm - on recombinant peptides for islet encapsulation and beta cell delivery
- HCM Medical - on GMP Critical CO_2 sterilization methods for cell delivery devices
- Visualsonics – on live imaging in small animal models
- Medace – on iso certified QMS and clean room production of cell delivery devices for clinical use

Main grants
Total amount (MERLN part)
- JDRF 3-SRA-2016-256-S-B (2016-2021); $ 899,999 ($ 899,999)
- JDRF 3-SRA-2017-428-S-B (2017-2020); $1,050,000 ($1,050,000)
- RegMed XB Diabetes moonshot (2017-2022); € 2,800,000 (€ 956,000)
- TKI-LSH diabetes moonshot scale up (2020-2022); € 27,000 (€ 27,000)
- NWO ENW-XS grant (2020-2021); € 50,000 (€ 50,000)
- Rubicon fellowship grant (2020); € 101,790 (€ 101,790)
- Albert Renold travel grant (2019); € 7,875 (€ 7,875)
- FRH/BD/145765/2019 Fundacáo Portuguesa para a Ciência e Tecnologia (2020); PhD scholarship grant (€ 0)
- Brightlands innovation award (2017); € 10,000 (€ 10,000)

Selected publications
- Reys, L. L., **Vaithilingam, V., Sthijns, M. M. J. P. E., Soares, E., Rademakers, T., de Vries, R., Mohammed, S., de Bont, D., Jetten, M., Hermanns, C., da Silva Filho, O., Stell, A.,** Mourad, N. I., Gianello, P**., LaPointe, V. L. S.,** Silva, S. S., Reis, R. L., Silva, T. H., **van Apeldoorn, A.** (2021). Fucoidan hydrogels significantly alleviate oxidative stress and enhance the endocrine function of encapsulated beta cells. Advanced Functional Materials, In Press. https://doi.org/10.1002/adfm.202011205
- **Sthijns, M. M. J. P. E., Jetten, M. J., Mohammed, S. G., Claessen, S. M. H., de Vries, R. H. W., Stell, A., de Bont, D. F. A.,** Engelse, M. A., Mumcuoglu, D., **van Blitterswijk, C. A.,** Dankers, P. Y. W., de Koning, E. J. P., **van Apeldoorn, A. A., LaPointe, V. L. S.** (2021). Oxidative stress in pancreatic alpha and beta cells as a selection criterion for biocompatible biomaterials. Biomaterials, 267, [120449]. https://doi.org/10.1016/j.biomaterials.2020.120449
- Hadavi, E., **de Vries, R. H. W.,** Smink, A. M., de Haan, B., Leijten, J., Schwab, L. W., Karperien, M. H. B. J., de Vos, P., Dijkstra, P. J., **van Apeldoorn, A. A.** (2021). *in vitro* degradation profiles and *in vivo* biomaterial–tissue interactions of microwell array delivery devices. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 109(1), 117-127. https://doi.org/10.1002/jbm.b.34686
- Skrzypek, K., Groot Nibbelink, M., Liefers-Visser, J., Smink, A. M., Stoimenou, E., Engelse, M. A., de Koning, E. J. P., Karperien, M., de Vos, P., **van Apeldoorn, A.,** Stamatialis, D. (2020). A High Cell-Bearing Capacity Multibore Hollow Fiber Device for Macroencapsulation of Islets of Langerhans. Macromolecular Bioscience, 20(8), [2000021]. https://doi.org/10.1002/mabi.202000021
- Hadavi, E., Leijten, J., Engelse, M., de Koning, E., Jonkheijm, P., Karperien, M., **van Apeldoorn, A.** (2019). Microwell Scaffolds Using Collagen-IV and Laminin-111 Lead to Improved Insulin Secretion of Human Islets. Tissue Engineering. Part C. Methods , 25(2), 71-81. https://doi.org/10.1089/ten.tec.2018.0336
- Hadavi, E., Leijten, J., Brinkmann, J., Jonkheijm, P., Karperien, M., **van Apeldoorn, A.** (2018). Fibronectin and Collagen IV Microcontact Printing Improves Insulin Secretion by INS1E Cells. Tissue Engineering. Part C. Methods , 24(11), 628-636. https://doi.org/10.1089/ten.tec.2018.0151
- Nibbelink, M. G., Skrzypek, K., Karbaat, L., Both, S., Plass, J., Klomphaar, B., van Lente, J., Henke, S., Karperien, M., Stamatialis, D., **van Apeldoorn, A.** (2018). An important step towards a prevascularized islet microencapsulation device: *in vivo* prevascularization by combination of mesenchymal stem cells on micropatterned membranes. Journal of Materials Science-Materials in Medicine, 29(11), [174]. https://doi.org/10.1007/s10856-018-6178-6

Selected outreach activities
- Regional radio interview L1: JDRF type 1 diabetes research, 2016.
- Regional radio interview Maastricht Radio, 2016.
- National radio interview “Met het oog op Morgen” Radio 1, Diabetes moonshot Interview, 2017.
- Interview in Telegraaf, a national newspaper on diabetes technologies within RegMed XB, 2017.
- Interview Hecht (MUMC+) magazine on Type 1 diabetes research, 2017.
- Invited lecture Diabetes fonds (Diabetes Fund Health Organization) patient info evening, Maastricht 2017.
- Invited lecture JDRF walk, a fundraising event for type 1 diabetes research including interactive discussions with scientists and patients on research and disease related topics, Lisserbroek, 2018.
- Invited lecture at PAS Festival Maastricht on treating type 1 diabetes with implants, Maastricht, 2017.
- Invited lecture at Stichting DON (Type I Diatebes Health Foundation) fundraising dinner, Wijnegem, Belgium, 2019.

Case study 9:

Reproductive medicine

Background
About 15 to 20% of all pregnancies abruptly end in a miscarriage and 1 out of 100 women even have to suffer recurrent miscarriage. Most of the miscarriages happen in the very early phase of pregnancy until the 12th week and the cause of a miscarriage is often unknown. Despite the great methodological advances in molecular and cell biology, our knowledge about early embryo development is still very limited. This is mainly due to the small size of the embryo and the inaccessibility of the embryo in the womb. This knowledge is however crucial as minor flaws at the beginning of a pregnancy can be the reason for miscarriages but can also lead to infertility or contribute to diseases during adult life. The field of stem cell research brings new hope to shed more light on the intricate process of early embryonic development. Engineering synthetic embryos, such as our blastoid model (Rivron et al., Nature 2018, Vrij et al., BioRxiv 2019), emerged as a promising new research field that has the potential to increase our fundamental knowledge about embryonic development and implantation, provide innovative embryo models for novel ways of experimentation (Samal et al., Advanced Materials, 2019), such as screening applications, and may provide new routes for improving success in reproductive medicine.

MERLN's approach

In one of our projects in the synthetic embryo field, funded by the Stichting de Weijerhorst, we are investigating the induction, self-organization and behaviour of the three founder cell types of the mouse embryo and how they interact with each other. Building upon the blastoid models (Rivron et al., Nature 2018), we have identified a combination of signalling pathway modulators that efficiently induces extraembryonic endoderm cells, which is one of the three founder cell types which was not present in our initial blastoid model (Vrij et al., BioRxiv 2019). Exposing a defined combination of factors to naive embryonic stem cell aggregates allowed us to efficiently induce a free-standing, three-dimensional extraembryonic/epiblast-like niche. These structures then spontaneously progress within a hydrogel- and serum-free medium towards the post-implantation stage, including a visceral endoderm-like layer encasing a polarized epiblast with pro-amniotic-like cavity. Apart from improving the embryonic niche, we are focussing in this project also on a robust and optimized formation of an engineered and functional trophectoderm and the interaction of the embryonic and extra-embryonic cells with maternal tissues. The latter is performed in close collaboration with clinicians at the Woman, Mother and Child Center of MUMC+ and aims at the development of a microfluidic chip to study implantation of our synthetic embryos in a 3D model of a receptive endometrium *in vitro*. These studies will also include the development of a human implantation model, which will further improve the translational aspects of our fundamental work and will help us to further decipher the black box of post-implantation development to eventually develop improved treatments for repeated miscarriages and implantation failures (*in vitro* fertilization).

The development of synthetic embryos faithfully recapitulating early embryonic development is highly dependent on the development of customized and tailored culture and analysis platforms (3, 5). Next to our scientific goals, we therefore dedicate time and effort to devise new microengineered tools, which allow us to precisely dissect and control our 3D models *in vitro*. In this context, we have developed a new microengineered culture, manipulation and open-source analysis platform for synthetic embryos, which integrates 4D image acquisition, automated feature extraction and machine learning to analyse migration of single cells within the multicellular synthetic embryos (Samal et al., Advanced Materials 2020). Meanwhile, this platform has been further developed to analyse not only a few cells but all cells of a stem cell aggregate, such as a blastoid or gastruloid. Current efforts include further development of this microengineered platform to analyse complex and collective migratory patterns and to generate fate maps of cells in embryo models that are exposed to local sources and defined gradients of soluble factors in customized microfluidic chips.

The work on synthetic embryos poses a unique opportunity to gain new in-depth knowledge about early embryonic development. For example, the mouse blastoid, which was invented in our labs, will provide access to knowledge, which has not been accessible so far due to several ethical and technical limitations. The direct translation of this new fundamental knowledge in a close dialogue with clinicians will be vital in order to implement new approaches in clinical practice e.g. to treat miscarriages, to improve the yield of *in vitro* fertilization techniques and to make these treatments safer for patients in the future. Moreover, our active involvement in the ethical and societal debate about the moral and ethical status of synthetic embryos will further help in creating a better awareness in society about recent and ground breaking developments in the field of stem cells and will hopefully contribute to a better-educated society.

Key points

- We have determined defined conditions that allow epiblast progression to the post-implantation stage in our embryo models.
- We are currently working on controlled lineage inductions in synthetic embryos, dissecting communication between the main embryonic and extra-embryonic lineages and tracing the fates of these self-organizing stem cells *in vitro.*
- We have developed unique 4D image acquisition, automated feature extraction and machine learning to analyse migration of single cells within the multicellular synthetic embryos to elucidate mechanisms underlying morphogenesis.

Collaborations

Internal

- Prof. Guido de Wert and Dr Wybo Dondorp (UM, Reproductive and Perinatal Medicine) – on ethics of synthetic embryos
- Prof. Han Brunner, Dr Masoud Zamani Esteki and Dr Edith Coonen (MUMC+, Clinical Genetics) – on genetics of blastoids
- Dr Ron van Golde, Dr Andrea Romano, and Dr Aafke van Montfoort (MUMC+, Reproductive Medicine) – on *in vitro* implantation models
- Dr Willem Voncken (UM, Molecular Epigenetics) – on *in vitro* implantation models

National:

- Prof. Gert Jan Veenstra and Dr Hendrik Marks (Radboud University) – on development of various embryo-like structures
- Prof. Susana M. Chuva de Sousa Lopes (Leiden University Medical Center) – on development of various embryo-like structures
- Prof. Petra Verhoef (Rathenau Institute) – on societal aspects and impact of blastoid and synthetic embryo research

International:

- Prof. Guido Pennings (Ghent University, Belgium) – on the international perspectives on the ethics of blastoids and embryo-like structures

Industry:

- Nikon – on high-throughput imaging and deep-learning based automated analysis of embryo-like structures
- 300MICRONS – on 3D cell culture tools and microwell arrays for guided self-organization
- BioLamina – on extracellular matrix proteins for stem cell culture and guided self-organization
- Stem Cell Technologies – on stem cell culture and protocols

Main grants

Total amount (MERLN part)

- EU-H2020-ERC-ADV-2015 ERC Advanced grant van Blitterswijk ORCHESTRATE; Building complex life through self-organization: from organ to organism (2016-2020); € 2,655,000 (€ 2,655,000)
- Stichting De Weijerhorst, Synthetic Embryos (2019-2023); € 2,673,600 (€ 2,673,600)
- ZonMw Enabling Technology Hotel, Decoding effects of bioactive soluble factors on transcriptional programs in mouse embryonic stem cell-based embryoid bodies using high-throughput screening platforms. (2021-2022); € 29,966 (€ 29,966)

Selected publications

- **Rivron, N. C., Frias-Aldeguer, J., Vrij, E. J.,** Boisset, J-C., Korving, J., Vivie, J., **Truckenmüller, R. K.,** van Oudenaarden, A., **van Blitterswijk, C. A.,** Geijsen, N. (2018). Blastocyst-like structures generated solely from stem cells. Nature, 557(7703), 106-111. https://doi.org/10.1038/s41586-018-0051-0
- **Vrij, E. J., Scholte op Reimer, Y. S., Frias Aldeguer, J.,** Misteli Guerreiro, I., Kind, J., Koo, B., **van Blitterswijk, C. A., Rivron, N. C.** (2019). Chemically-defined induction of a primitive endoderm and epiblast-like niche supports post-implantation progression from blastoids. BioRxiv preprint, [510396]. https://doi.org/10.1101/510396
- **Samal, P., van Blitterswijk, C., Truckenmüller, R., Giselbrecht, S.** (2019). Grow with the Flow: When Morphogenesis Meets Microfluidics. Advanced Materials, 31(17), [1805764]. https://doi.org/10.1002/adma.201805764
- **Samal, P., Maurer, P., van Blitterswijk, C., Truckenmüller, R., Giselbrecht, S.** (2020) A New Microengineered Platform for 4D Tracking of Single Cells in a Stem Cell Based *in vitro* Morphogenesis Model. Advanced Materials, 32, [1907966]. https://doi.org/10.1002/adma.201907966

- **Shankar, V., van Blitterswijk, C., Vrij, E., Giselbrecht, S.** (2021). From Snapshots to Development: Identifying the Gaps in the Development of Stem Cell based Embryo Models along the Embryonic Timeline. Advanced Science, [2004250]. https://doi.org/10.1002/advs.202004250

Selected outreach activities
- "Regeneratieve Geneeskunde" lecture by Dr Erik Vrij, Elkerliek Hospital, Helmond, October 2019.
- Lecture on model embryos, Special session of Studium Generale "Gezondheidsuniversiteit verdiept" by Dr Erik Vrij, Maastricht, March 2019.
- Talkshow on regional television 1Limburg "AvondGasten": Leven creëren zonder bevruchting (Creating life without fertilization), on the ethical implications and scientific use of synthetic mouse embryos, with Prof. Guido de Wert, May 2018.
- Talkshow on national television "Pauw": Kunstmatige embryo's gemaakt uit stamcellen van muizen (synthetic embryos made from murine stemcells), Amsterdam, May 2018. https://www.bnnvara.nl/pauw/artikelen/vanavond-bij-pauw-140, https://www.npostart.nl/pauw/03-05-2018/BV_101385950?utm_medium=refferal&utm_source=tvblik

Case study 10:

NWO-funded gravitation program:

Materials-Driven Regeneration (MDR)

Background
Regenerative medicine (RM) is an emerging interdisciplinary field where materials science, cell biology, bioengineering and the medical sciences converge with the aim to regenerate damaged and diseased organs and tissues and restore their normal function. As such, RM is considered a translational research field, the success of which is judged by its clinical and societal impact. This is also reflected by the type of funding that is available for this type of research with the expected output at a high technology readiness level. MERLN has been and still is involved in numerous such projects, including the national RegMed XB program (see Case study 11), as well as a range of EC-funded projects (e.g., JointPromise, see Case study 12). A disadvantage of this positioning of the field is the fact that limited funding is available for fundamental research in the disciplines comprising RM, which is very much needed in order to ensure a continuous source of innovation. Indeed, our understanding of the fundamentals of cell, tissue and organ regeneration and of how to stimulate and guide this inside the human body is still in its infancy. For RM to live up to its promise, fundamental scientific breakthroughs are essential.

With this in mind, MERLN has, together with leading RM scientists at Eindhoven University of Technology (TU/e), and Utrecht University, University Medical Center Utrecht and the Hubrecht Institute (RMU), successfully applied for an NWO Gravitation grant in 2017, which is the largest source of national funding for curiosity-driven research. Building on their mutual strengths in materials science, cell and organoid biology and complex tissue engineering, and facilitated by an €18.8 M ten-year Gravitation grant, they established the Research Center for Materials-Driven Regeneration (MDR) - a research partnership with the overarching aim to advance RM by regenerating tissue and organ function through intelligent, life-like materials. Next to establishing an excellent, synergistic and long-standing research program that will push the frontiers of RM in the Netherlands and beyond, MDR aims to secure the Netherlands' scientific RM leadership by creating the strong knowledge infrastructure to pass the baton from today's RM frontrunners to future talent, finding and training the next generation of the field's leaders.

Solution
The central goal of MDR's research program is to investigate, design and use intelligent biomaterials that drive the functional regeneration of living tissues and organs under complex (patho)physiological conditions. In three main research Flagships, the program targets the dynamic reciprocity between functional, life-like materials and the biological processes of regeneration at hierarchical levels of increasing length scale and structural complexity: the cellular and organoid level (Flagship 1), the tissue level (Flagship 2) and the organ level (Flagship 3). The scientific insights gained are used for the rational design of novel, intelligent biomaterials that drive endogenous regeneration - i.e. inside the human body - of load-bearing musculoskeletal and cardiovascular tissues and inherent organ functions (mobility, contraction, and filtration and removal of waste products). The research is designed to maximise synergy and collaboration across institutes and Flagships. An Enabling Technologies Platform is installed to support the program and its researchers with top-notch technology and related expertise available at the institutes, whereas a Clinical Translation Platform is established to prepare for careful, efficient, and rewarding translation of the most promising research outcomes to regenerative therapies that will impact future medicine. While MDR's main focus is on fundamental knowledge gain, the consortium has spurred new initiatives and collaborations to enhance knowledge utilization and future clinical translation, such as with RegMed XB. MDR has developed an extensive talent development program, ranging from graduate course on RM which includes theoretical and practical training at the involved institutions, to fellowships for PhD candidates and postdoctoral researchers to establish collaborations and do a part of their project at another (international) lab.

MERLN's approach
MERLN's researchers are involved in all Flagships of the program. The group of Dr Aurélie Carlier pursues *in silico* strategies that integrate knowledge on the interaction between extracellular matrix molecules and cell-membrane receptors, such as integrins (see Case study 5). In the next stage, the integrin model will be extended with other cell membrane receptors to obtain fundamental biological understanding of how cells integrate multiple signals and how these can be combined in new material designs. Dr Matthew Baker is involved in a research line focusing on understanding the composition and properties of extracellular matrix and designing its synthetic alternatives. Prof. Pamela Habibovic and Dr Sabine van Rijt supervise researchers who work on understanding how synthetic bone graft substitutes with osteoinductive capacity can be rendered suitable for load-bearing situations, also in compromised patients, suffering from osteoporosis, diabetes mellitus, or with bone defects resulting from tumour removal. Prof. Roman Truckenmüller, Dr Stefan Giselbrecht and Prof. Pamela Habibovic co-lead a research line that focuses on building a model of enthesis (bone-tendon interface) to study the process of degeneration and regenerative approaches. En route to designing intelligent materials that guide kidney organoids or organoid-derived cells towards functional kidney units, Prof. Roman Truckenmüller and Dr Stefan Giselbrecht contribute to research focusing on understanding how heterogeneity develops and how the different renal cell types expand in organoids in order to improve maturation, direct cell behaviour and organization, and recapitulate physiological function necessary for therapeutic application.

Key points
- MERLN was one of the three main applicants of the first NWO Gravitation grant awarded to researchers in the field of RM, facilitating a 10-year fundamental research program.
- Together with RegMED XB program (Case study 11), we cover the entire range from early, curiosity-driven research to highly translational research and research valorization in the RM field in the Netherlands.

Collaborations
- Partners: Prof. Carlijn Bouten (Eindhoven University of Technology), Prof. Marianne Verhaar (Utrecht University and University Medical Center Utrecht), Prof. Hans Clevers (the Hubrecht Institute).

- Affiliates: Prof. Sander Leeuwenburgh (Radboud University Medical Center).
- MDR's educational and talent development activities are open to other RM researchers in the Netherlands and beyond.
- International Advisory Board: Prof. John Jansen (chair, Radboud University Medical Center), Prof. Viola Vogel (ETH Zürich, Switzerland), Prof. David Mooney (Harvard University, USA), Prof. Katarina Le Blanc (Karolinska Institute, Sweden), Prof. Joseph Bonventre (Harvard Medical School, USA).

Selected publications

- **Vermeulen, S., Roumans, N., Honig, F., Carlier, A., Hebels, D. G. A. J., Dede Eren, A.,** ten Dijke, P., **Vasilevich, A., de Boer, J.** (2020). Mechanotransduction is a context-dependent activator of TGF- signaling in mesenchymal stem cells. Biomaterials, 259, [120331]. https://doi.org/10.1016/j.biomaterials.2020.120331
- Vassey, M. J., Figueredo, G. P., Scurr, D. J., **Vasilevich, A. S., Vermeulen, S., Carlier, A.,** Luckett, J., **Beijer, N. R. M.,** Williams, P., Winkler, D. A., de Boer, J., Ghaemmaghami, A. M., Alexander, M. R. (2020). Immune Modulation by Design: Using Topography to Control Human Monocyte Attachment and Macrophage Dierentiation. Advanced Science, [1903392]. https://doi.org/10.1002/advs.201903392
- **Yao, T., Wieringa, P., Chen, H., Chandrakar, A., Samal, P., Giselbrecht, S., Baker, M. B., Moroni, L.** (2020). Fabrication of a Self-assembled Honeycomb Nanofibrous Scaffold to Guide Endothelial Morphogenesis. Biofabrication, 12, [045001]. https://doi.org/10.1088/1758-5090/ab9988
- **Yao, T., Chen, H., Samal, P., Giselbrecht, S., Baker, M. B., Moroni, L.** (2019). Self-assembly of electrospun nanofibers into gradient honeycomb structures. Materials & design, 168, 1-8. [107614]. https://doi.org/10.1016/j.matdes.2019.107614

Selected outreach activities

MDR outreach page: http://mdrresearch.nl/outreach/

Case study 11:

Public–private partnership Regenerative Medicine Crossing Borders (RegMed XB)

Background

Ageing and changing lifestyles are driving a rapid increase in the chronic disease burden around the world. In response, our healthcare system has been geared towards caring for the chronically diseased: relieving symptoms and slowing down disease progression. This system is labour-intensive and expensive. RM offers a powerful potential solution to a variety of chronic diseases, but our experience has taught us that new partnerships and approaches are needed to develop genuine cures that would be affordable and accessible to the patients in need. To address this need, MERLN took a leading role in establishing RegMed XB (www.regmedxb.com), a public–private partnership that aims to perform excellent research and quickly and optimally translate the results into patient solutions and new businesses.

Led by Prof. Clemens van Blitterswijk (Founder, Chairman of the Scientific Advisory Board), Prof. Marianne van der Steen (Founding Director, current New Business Director) and Dr Vanessa LaPointe (Deputy Director until 2020), we embarked on establishing this virtual institute and securing a € 250 million commitment from diverse partners. We brought together multiple health foundations, top scientists from Dutch and Belgian universities, entrepreneurs, and regional and national governments). Four "moonshots" were established to develop cures for cardiovascular disease, kidney disease, osteoarthritis, and type I diabetes. We also established a translational infrastructure to identify and foster business opportunities to develop an RM ecosystem in the Netherlands, as RM is not only a promise to patients; it is also a major economic opportunity. With active roles in public affairs and outreach, RegMed XB also aims to bring RM to the forefront of (national) strategic agendas.

Solution

RegMed XB has broad activities in research, public–private partnerships, outreach, and new business development. Here, the four "moonshots" aiming to perform excellent research to generate cures to chronic diseases are briefly described:

Cardiovascular disease. Many cardiac diseases result in the loss of cardiomyocytes, which ultimately leads to cardiac failures. There are approximately 150,000 patients with cardiac failure in the Netherlands. Our approach to help these patients is based on activating the endogenous regenerative capacity of cardiomyocytes. Our aim is to remove the diseased heart, repair it ex vivo, and transplant it back into the same patient.

Kidney disease. There is a worldwide epidemic of end-stage kidney disease. At present, dialysis and transplantation are the only treatment options, but dialysis is accompanied by high morbidity and mortality, and transplantation is limited by a shortage of available organs. In the Netherlands alone, 6,500 people currently depend on dialysis, approximately 1,300 of which will die this year. We aim to create transplantable kidney tissue, either from induced pluripotent stem cells or adult stem cells. These transplants could reduce the need for dialysis, eventually replacing it entirely.

Osteoarthritis. Knee osteoarthritis is the most common joint disease, affecting 250 million people worldwide. It involves the degeneration of knee joint cartilage and the underlying bone. There is no single known cause of osteoarthritis, with diverse risk factors including previous injury, abnormal joint anatomy, obesity, inherited genetic factors, and ageing. Patients can experience severe pain accompanied by a decreased range of motion and reduced mobility. We are currently working on the steps towards a biological joint replacement, a combination of cells and materials that can be used to replace a small or large joint.

Type I diabetes. In this disease, the insulin-producing cells in the islets of Langerhans of the pancreas are destroyed by an autoimmune reaction. The consequence for the 150,000 people in the Netherlands with this disease is the need for lifelong insulin injectors, but they are at a high risk for serious complications such as renal failure or blindness. Our aim is to bring a new therapy for type I diabetes into a human trial, involving pancreatic cells that can produce insulin in response to blood sugar without the need for immunosuppressive agents.

MERLN's approach

In addition to taking a leading role in establishing RegMed XB, MERLN also plays an important scientific and leadership role. Prof. van Blitterswijk is the Chairman of the Scientific Advisory Board and Prof. van der Steen is the New Business Director and member of the Board of Directors. Researchers from all three MERLN departments are involved, and leadership positions within the moonshots are held by Dr Vanessa LaPointe (Kidney) and Dr Aart van Apeldoorn (Diabetes). Furthermore, Dr Baker and Prof. Lorenzo Moroni supervise researchers in the Cardiovascular Moonshot, Dr Matt Baker, Dr Aurélie Carlier, Dr Stefan Giselbrecht and Prof. Roman Truckenmüller supervise researchers in the Kidney Moonshot, and Prof. Pamela Habibovic supervises researchers in the Osteoarthritis Moonshot. This large team brings in diverse expertise and methodology, especially on biomaterials, cell biology, and tissue engineering.

Key points

- MERLN took a leading role in establishing this large public–private partnership in the RM field, involving academic partners, (local) governments, health foundation and industry, which is first of its kind in the Netherlands.
- RegMed XB has succeeded in forming the RM ecosystem in the Netherlands, having aligned public and private partners and built an infrastructure for the translation of scientific breakthroughs.

Collaborations
The success of RegMed XB is due to the commitment of diverse set of stakeholders, who together helped develop and establish the institute.

- Health foundations: Dutch Kidney Foundation, Dutch Diabetes Research Foundation, ReumaNederland, Dutch Cardiovascular Alliance and the Dutch Heart Foundation, Diabetes Onderzoek Nederland.
- Universities and medical centers: LUMC (e.g., the groups of Prof. Ton Rabelink and Prof. Eelco de Koning), UU/UMCU (e.g., the groups of Prof. Marianne Verhaar and Prof. Pieter Doevendans), TU/e (e.g., the groups of Prof. Carlijn Bouten and Prof. Keita Ito).
- Governments: Dutch and Flemish ministries and regional governments
- Companies: Fujifilm, LifeTec Group, Scinus, Suprapolix, Veldlaser, and many others. See www.regmedxb.com for a complete list.

Selected publications
- **Geuens, T., van Blitterswijk, C. A., LaPointe, V. L. S.** (2020). Overcoming kidney organoid challenges for regenerative medicine. NPJ Regenerative Medicine, 5, [8]. https://doi.org/10.1038/s41536-020-0093-4
- **Sthijns, M. M. J. P. E., Jetten, M. J., Mohammed, S. G., Claessen, S. M. H., de Vries, R. H. W., Stell, A., de Bont, D. F. A.,** Engelse, M. A., Mumcuoglu, D., **van Blitterswijk, C. A.,** Dankers, P. Y. W., de Koning, E. J. P., **van Apeldoorn, A. A., LaPointe, V. L. S.** (2021). Oxidative stress in pancreatic alpha and beta cells as a selection criterion for biocompatible biomaterials. Biomaterials, 267, [120449]. https://doi.org/10.1016/j.biomaterials.2020.120449
- Leuning, D. G., Witjas, F. M. R., Maanaoui, M., de Graaf, A. M. A., Lievers, E., **Geuens, T.,** Avramut, M. C., Wiersma, L. E., van den Berg, C. W., Sol, W. M. P. J., de Boer, H., Wang, G., **LaPointe, V.,** van der Vlag, J., van Kooten, C., van den Berg, B. M., Little, M. H., Engelse, M. A., Rabelink, T. J. (2019). Vascular bioengineering of scaffolds derived from human discarded transplant kidneys using human pluripotent stem cell-derived endothelium. American Journal of Transplantation, 19(5), 1328-1343. https://doi.org/10.1111/ajt.15200

Selected outreach activities
- Samenwerkende Gezondheidsfondsendag (Collaborative Health Foundations Day), Dr Tom Mastenbroek, November 2017. (https://www.hdmt.technology/event/7439/SFG-dag-2017-in-Dutch-)
- TEDx talk Prof. Clemens van Blitterswijk "Future of Regenerative Medicine", October 2017. (https://www.ted.com/talks/clemens_van_blitterswijk_the_future_of_regenerative_medicine)
- Nationale wetenschapsdagen (National sciences days), Prof. Ton Rabelink and Prof. Marianne Verhaar, 2020.
- Article in national newspaper Algemeen Dagblad on cardiovascular moonshot, February 2020. (https://www.ad.nl/gezond/primeur-eerste-hartoperatie-uitgevoerd-buiten-het-lichaam~a12da180/)
- Universiteit van Nederland (University of the Netherlands), Prof. Carlijn Bouten, "Hoe kun je van plastic levende hartkleppen maken" (How to make plastic heart valves alive), 2019. (https://youtu.be/if-fc42_IEg)
- The overall participation of patients in the moonshots, on all levels – e.g., evaluation of moonshot progress by patients.

Case study 12:

EU-funded program JointPromise

Background
Osteoarthritis is the most common chronic joint disease worldwide. For over 25% of the adult population it affects, it means suffering from pain and progressive loss of joint mobility. As a consequence, the disease is one of the leading causes of disability. This is a problem that is only becoming more serious with our ageing and increasingly obese population.

There are a variety of treatment options, including lifestyle changes and the use of painkillers or other medicines, but these do not slow down or reverse the progression of the disease. Eventually a joint replacement surgery is the only option. While these surgeries are commonly performed (e.g., 100,000 knees are replaced every year in the EU), the joint implants on average are satisfactorily functional for approximately 10 years, after which additional surgeries are needed. Moreover, synthetic joint implants, which are often metallic, often damage the surrounding bone tissue, leading to sub-optimal clinical outcomes.

Recently, there have been some promising breakthroughs in regenerative joint therapies, which may become an efficient alternative to synthetic joint implants. Today, it is possible to create biological living implants that can be used to replace the damaged bone and cartilage, effectively curing the joint of its osteoarthritis. However, a major challenge remains before patients can benefit from these breakthroughs, which is to make such therapies affordable and accessible to the many patients in need worldwide.

Solution
Led by Prof. Frank Luyten (KU Leuven), the JointPromise consortium (www.jointpromise.eu) was founded to bring a RM breakthrough from the lab into a setting where it can be manufactured under controlled quality systems. Funded by the H2020 work program (H2020-SC1-BHC-07-2019), JointPromise had a total budget of ~M€ 8, with ~M€ 1.7 for the MERLN Institute. The aim is to generate an implant composed of cells that are first assembled into small "microtissues" and are then further assembled into a construct that could replace the defect in the joint. The consortium is creating a robotically-driven pipeline that can generate the microtissues, to integrate a comprehensive quality control toolbox, and to use bioprinting technology to prepare the implants. This is a topic of significant strategic important to the MERLN Institute, as demonstrated by our successful application this year to the National Growth Fund (*Groeifonds*) to establish a RM manufacturing platform in the region.

MERLN's approach
MERLN is involved at multiple levels in JointPromise. We participate as a Work Package leader (Dr Vanessa LaPointe) and three PIs are supervising personnel in the project (Dr LaPointe, Dr Carlos Mota, and Dr Sabine van Rijt) from all three MERLN departments. Briefly, our contributions are as follows:

Dr Mota leads the bioprinting efforts to create an osteochondral implant. Pre-differentiated "microtissue" precursors of bone and cartilage are being combined with natural– and synthetic-based hydrogels to produce, by means of pressure-driven extrusion bioprinting, a dual construct implant. Furthermore, a hybrid approach is being investigated to introduce a vascular network to the bone compartment to ensure the survival of the implant. Sacrificial 3D printing will be investigated to achieve large vascular network. We aim for a final construct comprising the vascularized bone section and the cartilage layer.

Dr LaPointe and Dr van Rijt collaborate on a comprehensive quality control toolbox. Based on the European Medicines Agency's guidelines, which state that the process must be shown to perform effectively and reproducibly in meeting its predetermined specifications and quality attributes, they are collaborating with other partners on developing quality control measures. Multiple complementary methods are needed to comprehensively cover the different stages of production, and measurements that can take place during manufacturing should be complemented by destructive techniques that can provide more in depth information from a sample in the manufacturing pipeline. Due to the inherent variability in autologous therapies, they work to develop the knowledge and technologies to follow the progression of the "microtissues" and develop validated release criteria that can be used to obtain market authorization for the therapy.

Dr van Rijt's lab also develops nanotechnology-based tools for imaging and drug delivery. In this project, nanoparticles are designed to support the quality control aims. This is done by optimizing them to contain high imaging capabilities combined with the ability to bind specifically to identified quality control targets. For example, we are currently developing nanoparticle-based probes that can quantify extracellular matrix production within the cultured microtissues. This is an important quality control parameter to determine whether the microtissues matured sufficiently. The nanoparticle design allows modifications so that other targets can be imaged as well. The advantage of these tools is that they have an internal standard, so the target can be quantified, and that they can be used in real-time live imaging set-up.

Key points

- An example of an interdepartmental collaboration, where scientists from all three of MERLN's departments are working together for the aims of the project.
- An example of two key MERLN technologies (bioprinting and nanotechnology-based tools) being used in a pre-clinical translational pipeline with the intention to move on to clinical trials.

Collaborations

- Prof. Frank Luyten, Dr Ioannis Papantoniou, Prof. Liesbet Geris (KU Leuven, Belgium)
- Bastian Niessing, Jelena Ochs (Fraunhofer Institute, Germany)
- Dr Maria Klapa, Dr Nikolaos Moschonas (FORTH, Greece)
- Companies: Poietis (France), STEMCELL Technologies (UK)

Selected publications

- **Rosenbrand, R., Barata, D., Sutthavas, P.,** Mohren, R., Cillero-Pastor, B., **Habibovic, P., van Rijt, S.** (2018). Lipid surface modifications increase mesoporous silica nanoparticle labeling properties in mesenchymal stem cells. International Journal of Nanomedicine, 13, 7711-7725. https://doi.org/10.2147/IJN.S182428
- **van Rijt, S., Habibovic, P.** (2017). Enhancing regenerative approaches with nanoparticles. Journal of the Royal Society Interface, 14(129), [20170093]. https://doi.org/10.1098/rsif.2017.0093
- **van Rijt, S. H.**, Bölükbas, D. A., Argyo, C., Wipplinger, K., Naureen, M., Datz, S., Eickelberg, O., Meiners, S., Bein, T., Schmid, O., Stoeger, T. (2016). Applicability of avidin protein coated mesoporous silica nanoparticles as drug carriers in the lung. Nanoscale, 8(15), 8058-8069. https://doi.org/10.1039/C5NR04119H
- **van Rijt, S. H.**, Bölükbas, D. A., Argyo, C., Uhl, F., Burgstaller, G., Datz, S., Lindner, M., Eickelberg, O., Königshoff, M., Bein, T., Meiners, S. (2015). Protease-responsive nanoparticles enable specific targeted drug delivery in ex vivo lung tumors. ACS Nano, 9(3), 2377-2389. https://doi.org/10.1021/nn5070343
- **Mota, C., Camarero-Espinosa, S., Baker, M. B., Wieringa, P., Moroni, L.** (2020). Bioprinting: From Tissue and Organ Development to *in vitro* Models. Chemical Reviews, 120(19), 10547-10607. https://doi.org/10.1021/acs.chemrev.9b00789