Final Report SAW Professorinnenprogramm BIOMAT

Project title: W2-tenure track professorship as a joint appointment of DWI – Leibniz Institute and RWTH Aachen University

Project number: P62/2017

This report cannot be published yet as many results are not yet published in peer-reviewed papers. The content has to be kept confidential until the work is published.

Executive Summary

In BIOMAT, the main technology to be developed is a magnetic field-assisted (MFA) three-dimensional (3D) bioprinting technology to create 3D macroscopic constructs, with internal structures comparable to the nano- and microscale fibrillar extracellular matrix (ECM). Recapitulating the architecture of a desired *ex vivo* tissue is crucial to obtain functional tissue. In Objective 1.1, a bioink based on our Anisogel technology is developed to mimic the anisotropy of the tissues, guide cell growth, and direct functional tissue maturation. The hybrid ink contains micron-scale, magneto-responsive anisometric elements and a surrounding printable synthetic hydrogel precursor solution. Two poly (ethylene glycol) (PEG)-based synthetic bioinks are developed and synthesized, focused on Michael-type addition. The first ink crosslinks by mixing inside a custom-made microfluidic printing nozzle. In the second ink, all components can be mixed as the reactive crosslinker is protected with a photoprotective group (PPG). The Anisogel-based bioink resulted in multi-layered printed constructs, where the direction of the magneto-responsive microgels is altered in each layer by turning the magnets on the printbed. In addition, we can now use one static magnetic field, as the rod-shaped microgels can be rendered magnetic with pre-aligned ellipsoidal magnetic particles during their production, which allows us to pre-program the angle at which angle the microgels will align compared to the field lines.

In Objective 1.2, we tested the growth, alignment and differentiation of mesenchymal stromal cells inside the Anisogel to induce chondrogenesis. Although there are a few regenerative treatments available that achieve the formation of cartilage-like tissue, this newly formed tissue does not recapitulate the native zonal differences in ECM composition and alignment. In this work, we optimized a PEG-based Anisogel for chondrogenesis of mesenchymal stromal cells (MSCs) by analyzing cell morphology and the deposition of cartilaginous markers such as collagen type II, or glycosaminoglycan (GAG). By using the Anisogel, directed cell growth and, thereby, oriented tissue formation was achieved.

In Objective 1.3, we included cell actuation inside our constructs. Microgels, made from poly (N-isopropyl acrylamide/N-ethyl acrylamide) (star-PNIPAm/NEAm) with plug-flow microfluidics at different gelation temperatures are used as photothermal actuators by adding gold nanorods (AuNRs) with a plasmon resonance at around 808 nm into the network. Irradiation with a near infrared laser is absorbed by the AuNRs resulting in a localized increase in temperature in the surrounding microgel. The polymer chains are heated above their lowest critical solution temperature (LCST), which causes the whole network to collapse. This collapse is used to create mechanical forces that stimulate cells. The transduction of force on cells in a larger 3D construct is tested by developing a microgel-in-hydrogel system, where a soft surrounding PEG-based hydrogel matrix is covalently bound to the thermoresponsive microgels. This system employs NIR light induced actuation of the microgels to create a pull on the hydrogel chains, which distributes the force evenly throughout the network, potentially influencing cells which are not directly in contact with the microgels themselves.

In Objective 1.4, we further optimized the hydrogels for nerve growth. Three types of nerve cells were tested in the PEG-based hydrogel, modified with bioactive domains: primary neurons from chick embryonic dorsal root ganglia (DRG) and mouse DRGs, and neurons derived from induced pluripotent stem cells. In addition, basic studies were performed in 2D and 3D to investigate the effect of the dimensions, aspect ratio, and spacing of the guiding elements on cell behavior. These studies were crucial to determining the threshold values to reduce the amount of synthetic material and magnetic nanoparticles, which are both important parameters to be minimized when used *in vivo*. In addition, the effect of fiber topography and distance on nerve growth was also studied. Finally, initial 2D experiments were performed to test the effect of photothermal hydrogel actuation on differentiation of muscle cells and mesenchymal cells. After this, we started investigating an actuating microgel-in-hydrogel system to study the effect of local mechanical actuation on cell behavior in 3D.

1. Achievement of objectives and milestones

Objective 1.1: MFA-Bioprinter development

Two PEG-based bioinks were developed and synthesized, both crosslink via Michael-type addition. In the first approach, two components are kept separate in a custom-designed microfluidic printing nozzle, consisting of two chambers and a mixer, to mix both components right before deposition on the printing bed. In the second approach, the donor molecule of the Michael-type addition has a photoprotective group (PPG), so that both donor and acceptor can be pre-mixed without them reacting with each other. In Figure 1, magnetic rods are printed inside the PEG bioink in 2 different directions. Cell spheroids spread into the printed constructs by controlling the degradation rate of the construct with two different crosslinkers, one sensitive to matrix metalloproteases and one to hydrolysis. This work is part of two papers in preparation: *'Synergizing Bioprinting and 3D Cell Culture to Enhance Tissue Formation in Synthetic Printed Constructs'* and *'Free Light-Activated Magneto-Responsive Bioink for 3D Bioprinting of Large Tissue Constructs'*

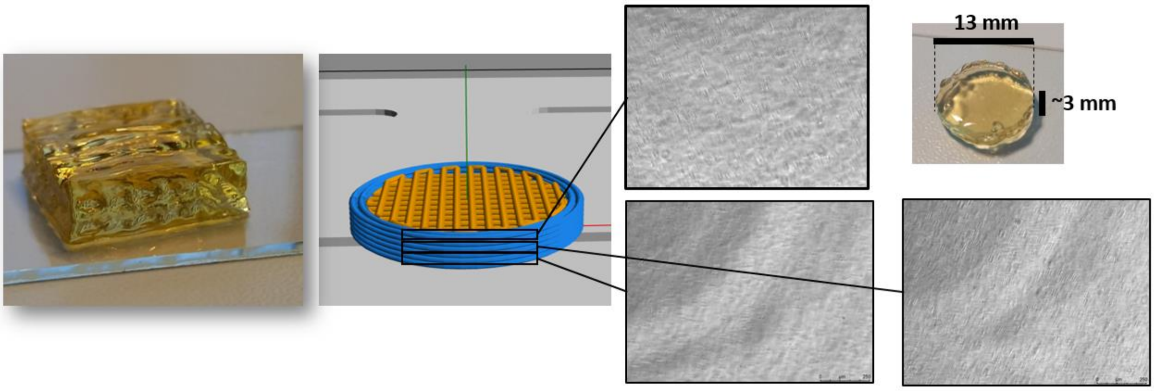

Figure 1| Printed 3D construct with MFA-bioprinting and an Anisogel-based bioink using anisometric magneto-responsive microgels (2.5 x 2.5 x 25 µm).

Other papers published in Objective 1.1 are:

Rose JC, Fölster M, Kivilip L, Gerardo-Nava JL, Jaekel EE, Gehlen DB, Rohlfs W*, **De Laporte L***. Predicting the orientation of magnetic microgel rods for soft anisotropic biomimetic hydrogels. Polymer Chemistry 2020, **11:** 496

Braunmiller DL, Babu S, Gehlen DB, Seuß M, Haraszti T, Falkenstein A, Eigen J, **De Laporte L***, Crassous J*. Pre-programmed rod-shaped microgels to create multi-directional Anisogels for 3D tissue engineering. Advanced Functional Materials 2022, 32: 2202430.

Objective 1.2: Cartilage Repair

To engineer functional cartilage, the generated tissue must resemble the native structural organization. To achieve this, we have embedded human mesenchymal stem cells and rod-shaped magneto-responsive microgels into our 3D hydrogel. Different microgel dimensions are tested (2.5x2.5x25 µm^3, 5x5x50 µm^3, and 10x10x100 µm^3) with different surrounding hydrogel stiffness (Figure 2). We observe better cell growth in lower weight percentage hydrogels and better cell alignment with microgels of the highest dimension 10x10x100 µm^3. This work is part of two papers in preparation: "*Promoting Zonal Organization in PEG Anisogel for Cartilage Regeneration*" and "*PDGF-BB and TGF-β1 to Promote Chondrogenesis of Mesenchymal Stromal Cells*".

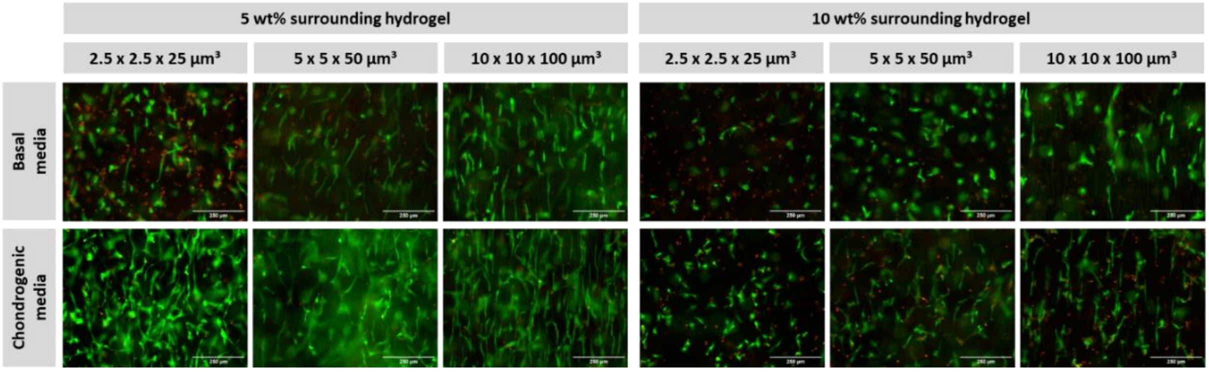

Figure 2| Effect of microgel alignment on MSC morphology and orientation. Scale bar 250 µm.

Other papers published in Objective 1.2 are:

Rose JC, Gehlen DB, Haraszti T, Köhler J, Licht CJ, **De Laporte L**. Biofunctionalized aligned microgels to provide 3D cell guidance to mimic complex tissue matrices. Biomaterials, 2018, 163: 128

Krüger AJK*, Bakirman O*, Guerzoni LPB, Jans A, Gehlen DB, Rommel D, Haraszti T, Kuehne AJC, **De Laporte L**. Compartmentalized jet polymerization as high-resolution process to continuously produce anisometric microgel rods with adjustable size and stiffness. Adv Mater. 2019, e1903668

Omidinia Anarkoli A, Ephraim J, Rimal R, **De Laporte L**. Influencing linear neurite extension via hierarchical fibrous guiding cues at different scales. Acta Biomaterialia 2020, 113: 350-9

Objective 1.3: Hydrogel actuation and TFM

As alternative to magnetic mechanical stimulation, we developed a material system based on photothermal microgel-in-hydrogel actuation. With a modulated laser, a pulsed irradiation of up to 2 Hz with varying on and off times is possible exerting cyclic forces on cells in a 3D environment (Figure 3). This work is part of the following paper in preparation: "*Thermally-assisted microfluidics to produce chemically equivalent microgels with tunable network morphology*"

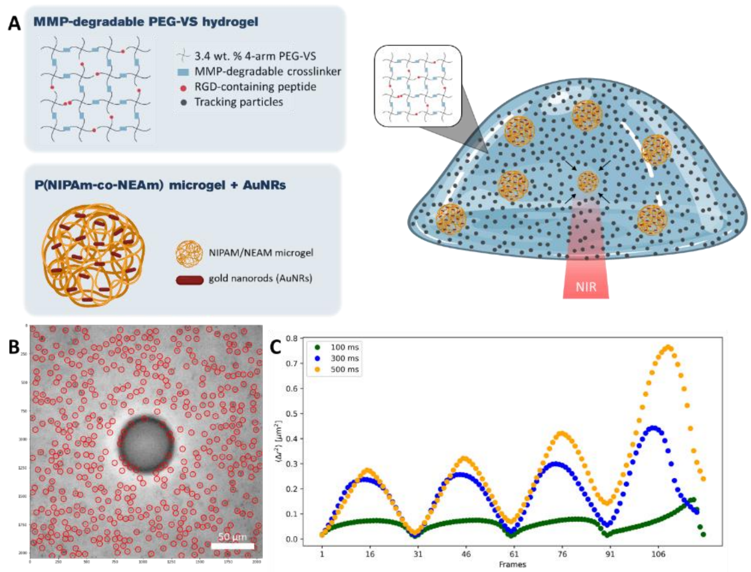

Figure 3| (A) Hydrogel matrix composition and schematic depiction of the photothermal actuation process during NIR irradiation. (B) Microgel-in-hydrogel during actuation (3.4 wt%, 0.1 eq. GMA, 1x10^6 beads/µL, 2.8 microgels/µL). Red circle represent tracking particles recognized by the python script for calculation of the mean squared displacement (MSD). (C) Comparison of MSD over the frames for a microgel irradiated at 100 ms, 300 ms, and 500 ms ON-time and 1 Hz frequency with a NIR-laser.

Objective 1.4: Cell mechanostimulation

2D and 3D platforms are employed to investigate the effect of the dimensions, aspect ratio, and spacing of the guiding elements on cell behavior. The 2D light-actuating hydrogel film, made from p(NIPAM-*co*-NEAM) and embedded AuNRs, was employed to actuate myoblasts and mesenchymal stem cells grown on top of the hydrogel film, demonstrating mechanically-stimulated early differentiation processes.

Published papers in Objective 1.4 are:

Vedaraman S, Perez-Tirado A, Gerardo-Nava J, Haraszti T, Nishiguchi A, **De Laporte L.** Anisometric microstructures to determine minimal critical physical cues required for neurite alignment. Adv. Healthcare Materials 2021, e2100874

Rose JC, Gehlen D, Omidinia Anarkoli A, Fölster M, Haraszti T, Jaekel E, **De Laporte L**. How much physical guidance is needed to orient growing axons in 3D hydrogels? Advanced Healthcare Materials 2020, 21: e2000886

Babu S, Chen I, Vedaraman S, Gerardo-Nava J, Licht C, Kittel Y, Haraszti T, Russo JD, **De Laporte L.** How do the local physical, biochemical, and mechanical properties of a synthetic Anisogel affect oriented nerve growth? Advanced Functional Materials 2022, 32: 2202468.

Chandorkar Y, Castro Nava A, Schweizerhof S, van Dongen M, Haraszti T, Köhler J, Zhang H, Windoffer R, Mourran A, Möller M, **De Laporte L**. Cellular responses to beating hydrogels to investigate mechanotransduction. Nat Commun. 2019, 10: 4027.

Chandorkar Y, Bastard C, Di Russo J, Haraszti T, **De Laporte L.** Cells feel the beat – Temporal effect of cyclic mechanical actuation on muscle cells. Applied Materials Today 2022, 27: 101492.

Castro Nava A*, Doolaar IC*, Labude N, Malyaran H, Babu S, Chandorkar Y, Di Russo J, Neuss S§, **De Laporte L§**. Actuation of soft thermoresponsive hydrogels mechanically stimulates osteogenesis in human mesenchymal stem cells without biochemical factors. ACS Appl. Mater. Interfaces 2024, 16: 30.

To investigate the mechanical stimulation in a 3D hydrogel, primary normal human dermal fibroblasts (NHDFs) and the GMA-functionalized NIPAm/NEAm microgels are embedded into a hydrogel, and alveolar cells are grown on the outside of actuating microgels.

This work is part of two papers in preparation: "*3D Pulsing Microgel-in-Hydrogel System to Mechanically Stimulate Cells*" and "*Actuating microgels to mimic alveolar movement in a 3D ex vivo lung model"*.

2. Activities and obstacles

Some different approaches are undertaken due to difficulties with the original plan.

- The first one is due to the fact that our synthesized PEG-vinylsulfonate seems to be toxic at higher concentrations, also after crosslinking. Therefore, we now synthesized linear and star-PEG-SH crosslinkers with hydrolysable esters instead.
- The second change is the position of the magnets in MFA-Bioprinting. Instead of adding them to the nozzle to pre-align the magneto-responsive elements before extrusion on the printbed, the magnets are now positioned on the printbed to align the elements after printing and before crosslinking of the ink.
- The third change is related due to the difficulties to rotate our magneto-responsive microgels inside visco-elastic gels. Therefore, we now focus on the alternative of using photothermal actuation of a microgel-in-gel system.

To avoid too many delays due to Covid 19, this time was used to write review papers:

Kittel Y, Kuehne AJC*, **De Laporte L***. Translating therapeutic microgels into clinical applications. Advanced Healthcare Materials 2021, e2101989.

Babu S, Albertine F, Omidinia-Anarkoli A, **De Laporte L**. Controlling structure with injectable biomaterials to better mimic tissue heterogeneity and anisotropy. Advanced Healthcare Materials 2021, 10: e2002221.

3. Results and successes

I currently have 74 peer-reviewed publications with an average impact factor of 10.1, 3 patents, and one patent pending. On an international level, I have presented at over 100 conferences, meetings and seminars (see CV, appendix 1). In addition, I am part of the Scientific Advisory Board of the journal Advanced Healthcare Materials, the Board of Directors of the International Society for Biofabrication ISBF, the Advisory Board of the European project ENLIGHT, coordinated by Utrecht University. During one year, I was an Associated Editor of the journal ACS Advanced Materials and Interfaces.

4. Equal opportunities, career development and internationalisation

At DWI, my research group has a high percentage of 46% international PhD students and Post-Docs and 54% female. This is supported by the many international EU projects (Biogel, ReWire, OrganTrans) and the participation in a large Canadian consortium. Besides my work at the RWTH, UKA, and DWI, I am a fellow of the Max Planck School 'Matter to Life'. To give other young international female researchers a platform to present their science, I initiated the Woman for Interactive Materials Award (WIMA), which took place the first time in 2021, and has since had 3 editions, with the aim to continue this on a yearly basis.

5. Structures and collaboration

The W2 tenure track professorship in the Chemistry Department started September 2018 with a Berliner model (100% DWI). Due to an external offer at an international university, the RWTH made a counter offer, including 20% RWTH, an early tenure was obtained in December 2020, including an affiliation with the Institute for Applied Medical Engineering at the University Hospital in Aachen. In March 2024, I received an offer from the RWTH to be promoted to W3 professor. These negotiations are ongoing.

With the material platforms we develop, my research group forms a bridge between natural science, engineering, and medicine, in line with the RWTH Excellence Strategy. I am currently part of several RWTH/UKA consortia (see CV Appendix 1): SFB985, KFO5011, GRK2415, GRK2375, and SPP2451. With DWI, I have coordinated a European Marie Skłodowska-Curie Innovative Training Network Biogel from 2015-2018 and since 2023 the EU Doctoral Network ReWire. ReWire is complementary to Mend the Gap, a large Canadian consortium I am part of, to establish a transformative biomaterials platform for spinal cord repair (funded by New Frontiers in Research Funds (NFRF) Transformations). In addition, I was part of OrganTrans, a large EU consortium to 3D print tissue-engineered liver constructs. Within Leibniz, I am active in the Leibniz Gesundheitstechnologien and part of the competence field Bioactive Materials. In our SAW Transfer, µTissue*fab* with INM (Saarbrücken) and IPF (Dresden), we aim to develop and translate multiphasic hydrogels for high-throughput human *in vitro* tissue and disease models. In a new lighthouse project TriggerINK with four other DWI PIs and funded by the Werner Siemens Foundation, we aim bioprint a regenerative construct directly *in vivo* for cartilage repair, which is a nice follow-up of the BioMAT project.

6. Quality assurance

All PhD students are obligated to attend "good scientific practice" course, organized by the Center of Doctoral Studies of the RWTH. Any digital and digitalizable data generated within this project are temporarily saved on institutional, non-private personal computers and ultimately secured on the backup servers of the DWI. We aim to mostly publish open access.

7. Additional resources

I have acquired significant funding from the DFG and from the EU, I was granted 5 ERC projects (StG, CoG, 3x PoC). (see CV, Appendix 1). For the co-financing of DWI, we paid part of the salary of the PI, part of a post-doc to work on medical devices, technicians to organize the cell culture laboratory and support chemical synthesis, and a bioprinter as large equipment.

8. Outlook

I aim to further develop transformative medical materials for both *in vitro* and *in vivo* tissue engineering. It is my dream to bring the injectable and alignable Anisogel into patients to regenerate part of the spinal cord after injury. To achieve this, the new building First in Translation offers a lot of opportunities to produce this material according to good manufacturing practice for potential investigator initiated clinical trials. With the standardized and automated high-throughput production of 3D *ex vivo* human mini-tissue, I aim to work closely with the pharmaceutical industry to provide more efficient alternatives for human therapy testing.