

TEDD Annual Meeting with 3D Bioprinting Workshop

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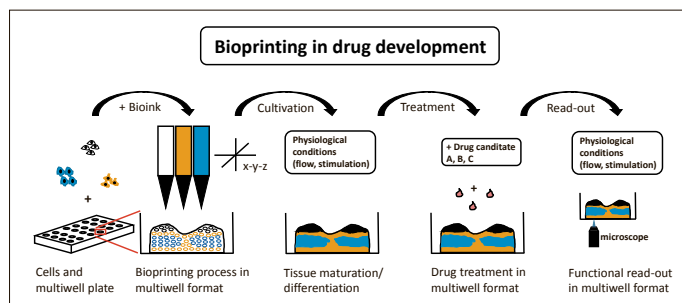
Abstract: Bioprinting is the technology of choice for realizing functional tissues such as vascular system, muscle, cartilage and bone. In the future, bioprinting will influence the way we engineer tissues and bring it to a new level of physiological relevance. That was the topic of the 2017 TEDD Annual Meeting at ZHAW Wädenswil on 8th and 9th November. In an exciting workshop, the two companies regenHU Ltd. and CELLINK gave us an insight into highly topical applications and collaborations in this domain.

Bioprinting opens up completely new perspectives, not only for creating complex tissue structures, but also as an efficient tool for drug discovery and preclinical testing. Introducing the topic, **Dr Markus Rimann**, Group Leader 3D Tissues and Biofabrication at ZHAW, provided an overview:

State-of-the-art of 3D Bioprinting for Drug Development

Over the last 10 years the technology has evolved quite rapidly as low-cost bioprinters have penetrated the market and became accessible to a broad scientific community. “One gets the impression that everybody is printing, sometimes without additional benefits compared to standard tissue engineering (TE) approaches. The technology is really being hyped up, the potential seems to be huge, but has to be verified”, argues Dr Markus Rimann. The leading bioprinting specialist thinks that printing of small tissues still has to prove it adds value compared to standard TE approaches. “Small tissues in well plates are suitable for drug development, and this is where bioprinting will make its first big impact. In the near future, when vascularized tissues are bioprinted in a robust manner, larger tissues will be fabricated, which will revolutionize regenerative medicine.”

For the time being, his group is conducting research on an industry-driven project to develop an integrated solution to address muscle diseases. The solution encompasses: 1) a specialized well plate with two posts in each well to 2) allow the bioprinting of human muscle cells and fixation of the developing muscle tissue between the posts and 3) electrical stimulation of the human muscle tissue in the well plate. The final goal is to monitor post bending after electrical muscle fibre stimulation with an optical force measurement device following drug exposure.



Simplified scheme of a potential scenario in drug development with bioprinting steps included. It is worth to mention that tissue production, maturation and read-out are carried out in the same well plate. Source: Markus Rimann, ZHAW.

In another research-driven project, the scientists are developing in assays that detect the presence of drug side effects on kidneys, termed nephrotoxicity “To gain experience in this area we developed a bioprinted tubular structure inside a hydrogel using sacrificial inks that are removed after the printing process. The hollow tubular structures are then seeded with proximal tubular epithelial cells (kidney) in one tube and endothelial cells (blood vessel) in the other tube. By doing so the scientists aim to represent the two communicating tubular structures in the kidney. Cell growth will take place under physiological conditions with perfusion over five days.” The expert on cell and molecular biology takes stock: “The final goal is to develop a functional assay based on receptor-mediated endocytosis to assess drug nephrotoxicity.”

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It Takes Two to Tango: The Promises of Hybrid 3D Bioprinting

With strategic support from his University, **Dr Nicanor I. Moldovan** has founded the 3D Bioprinting Core at Indiana University School of Medicine. He thinks that bioprinting is set to become one of the most disruptive next-generation bio-fabrication technologies. It aims to revolutionize tissue engineering and thus to improve the way we study disease mechanisms, discover new drugs or assess the effects of chemicals on living tissues, and will eventually change how we treat diseases. “So far, bioprinting mainly rode the wave of the additive manufacturing revolution, imitating the technically similar 3D printing”, says Moldovan, who is an Associate Research Professor at IUPUI (Indiana University – Purdue University Indianapolis). “However, this approach is dependent on the printability of the ‘scaffold’ biomaterial (bio-ink), which also has to accommodate and support live cells. In spite of the constant progress in this area, a universal bio-ink capable of serving all the requirements of the bioprinted constructs is still to be found. Therefore an increasingly sophisticated array of technological add-ons is incorporated into the

bioprinting process, some exploiting the resources of biomaterials, while others trying to minimize their involvement.” In his opinion, a radically different form of bio-fabrication would use only cells to create complex 3D tissue models. These scaffold-free methods would mostly rely on the properties of spheroidal cell aggregates. Accordingly, scaffold-free bioprinting methods have emerged that are capable of assembling multiple spheroids into larger construct. One of the most significant developments in this regard is the microneedle-assisted spheroids skewering –‘Kenzan’- method on the robot *Regenova* marketed by the company Cyfuse Biomedical (www.cyfusebio.com). “Our Core facility recently acquired one of the few such instruments outside Japan”, the scientist tells us. “However, scaffold-free bioprinting is not without its own limitations, and for this reason the field is looking back to the secondary incorporation of biomaterials within the workflow, as a form of ‘hybrid’ bioprinting.” In his keynote TEDD talk, Dr Moldovan discussed this and other forms of hybrid biofabrication.

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What Happens to Bioprinting in the Hands of a Dynamic Global Player?

Dr Maité Rielland knows, as she is currently leading the bioprinting platform at L’Oréal Advanced Research: “Since late 1980s, a long time before the European Union banned animal testing for cosmetic products in 2013, L’Oréal positioned itself as a pioneer in reconstructed human skin. It became one of the first cosmetic companies to test its raw materials/actives on in-house reconstructed human skin,” states Rielland, who has a Bachelor’s degree in Biology and specialized in stem cells during her PhD. “Bioprinting is a great complementary approach to creating new models of skin with a complexity that cannot be achieved by human hands alone.” The scientist knows what she is speaking about, as she carried out her postdoctoral fellowships at the Mount Sinai Medical Center and at New York University Medical Center investigating 2D and 3D *in vitro* models. “One of the biggest potential advantages of this technology is the ability to place cells or biological material where it needs to be placed, which opens a few doors for tissue engineering. And”, she adds, “it will become a tool for screening and model construction in the next few years, and it is already pushing us to think about 3D *in vitro* models and tissue engineering differently.”

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Bioprinting for Bone and Joint Regeneration

When it comes to bioengineering, **Prof. Daniel Kelly** is the person who pushes research findings into the clinic environment. He speaks from experience: “Our musculoskeletal system has a limited capacity for repair. This has led to increased interest in the development of tissue engineering strategies for the regeneration of musculoskeletal tissue such as bone, ligament, tendon, meniscus and articular cartilage. Today we are trying to use biomaterials and mesenchymal stem cells (MSCs) to tissue engineer functional articular cartilage and bone grafts for use in bone and joint regeneration.” Kelly is a Professor of Tissue Engineering at Trinity College Dublin and Director of the Trinity Centre for Bioengineering. As a founding principal investigator at AMBER, the new Advanced Materials and Bioengineering research centre



Dr Maité Rielland, Advanced Research Engineer, joined the tissue models team in L’Oréal’s Advanced Research organization in 2014 to establish new skin tissue models and install a bioprinting platform. Copyright © Matteo L’Oréal R&I.

based at Trinity College Dublin, he describes how 3D bioprinting can be used to engineer biological implants that mimic the shape of specific bones, and how these bioprinted tissues mature into functional bone organs upon implantation into the body. Due to his considerable skills in tissue engineering, he is able to demonstrate how different musculoskeletal injuries can be regenerated using 3D bioprinted implants, including large bone defects and osteochondral defects. At the end, he provided an overview showing how we can integrate biomaterials and MSCs into 3D bioprinting systems to engineer scaled-up tissues that could potentially be used to regenerate entire diseased joints.

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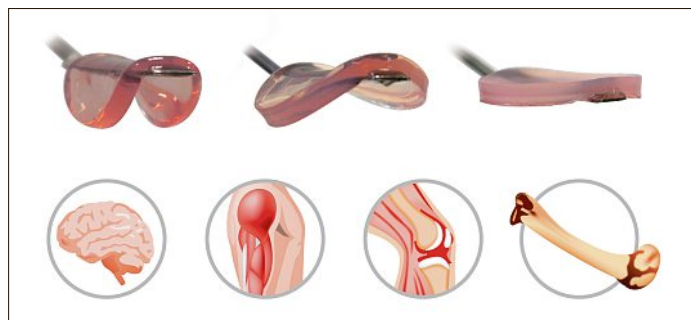
Developing Hydrogels for 3D Bioprinting

Hydrogels are 3D networks with a high water content and are widely used as cell carriers in tissue engineering because they have structural similarities to the natural extracellular matrix. Biogelx Limited in Scotland produces hydrogels made up of simple, short, yet biologically relevant peptides. “Our patented technology allows precise control over the mechanical and chemical properties of the gel”, says **Dr Laura Goldie**. She is responsible for Biogelx’s R&D portfolio, which includes both external and internal projects working on the development of peptide hydrogel technology for new applications. “The ability to create hydrogels of varying stiffness and chemical composition means that the matrix can be tuned to replicate the exact environment the cells need to grow, develop and thrive in a realistic *in vivo* manner”, she explains. “Biogelx gels are entirely animal-free, delivering a consistent uniformity. They offer a nanoscale fibre size with at least 99% water content. This replicates an *in vivo* environment for cells and allows, for example, the development of patient-specific, cell-based bench top systems.”

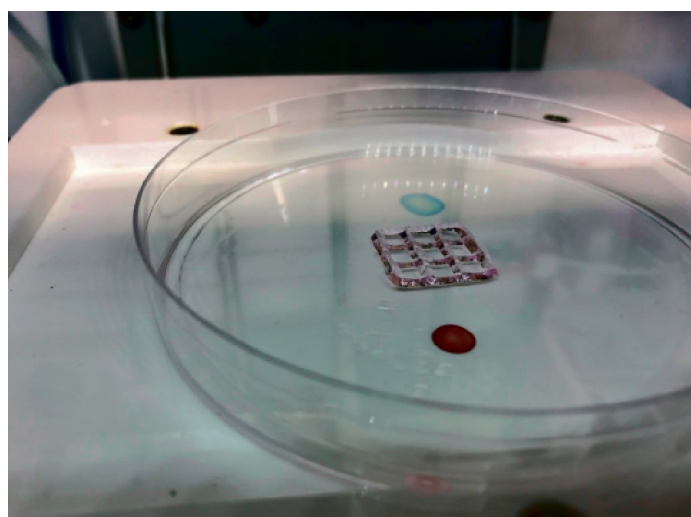
Biogelx cell-matched peptide gels are in use for stem cells to influence mesenchymal stem cell differentiation toward hard or soft tissue, cell phenotypes in the absence of differentiation media, immobilized matrix proteins or active matrix proteins. In cancer research, the 3D nature of the gels offers a major opportunity to develop more realistic *in vitro* models. In bioprinting, being able to control the mechanical and chemical properties of

Biogelx hydrogels offers the possibility of bio-inks with the potential to balance printability with cell viability.

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Example images of some of Biogelx's *in vitro* hydrogels, tuned to match different tissue environments. Source: Biogelx.



Lattice structure printed using Biogelx hydrogel. Source: Biogelx.



Biogelx hydrogel used in standard cell culture format. Source: Biogelx.

Workshop: This is How Bioprinting is Implemented

To give participants an extensive insight into their work, TEDD's long-time partners regenHU Ltd. and CELLINK held a half-day workshop at ZHAW Waedenswil, in which they demonstrated how they innovate by applying bioprinting.

regenHU Ltd. – The New Generation of Bioprinter 3DDiscovery® Evolution

regenHU Ltd. — a company in the three-dimensional biotechnology sector, that exploits the potential of 3D bioprinting & cell based therapies — is leading the development of biomedical products for regenerative medicine & drug discovery. Based in Fribourg, Switzerland, regenHU Ltd. is a pioneer and global leader in the field, converging digital manufacturing (3D printing), biomaterials and biotechnology to drive transformational innovations in healthcare.



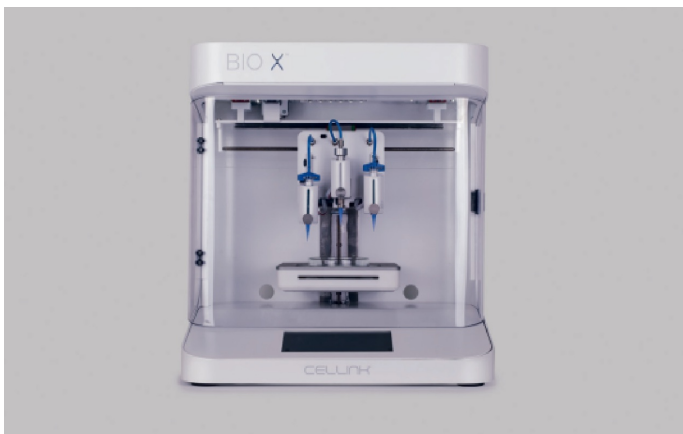
A special cooperation links the TEDD team – research associate Sandra Laternser (left), Dr Markus Rimann (right), Group Leader 3D Tissues and Biofabrication – to regenHU Ltd., a reliable partner in devising innovative ideas for applications in 3D bioprinting. Source: regenHU Ltd.

The company exploits new biomanufacturing solutions in order to respond to the emerging challenges facing the biomedical industry. Its last great breakthrough is the convergence of two key biofabrication technologies, bioprinting & electrospinning, in one single process unit. “Macro and nano dimensions in one single process unit are enabled with the 3DDiscovery™ Evolution, a powerful tool which creates tissue architectures comparable to those found in nature”, explains Marc Thurner, regenHU Ltd.'s founder and CEO. “That means we understand which stimuli have to be used and in what conditions, and we provide the scientific instrument to accomplish this.” 3DDiscovery™ Evolution has *modularity*: 11 different printhead technologies in a single instrument to optimize the instrument to your application needs, *flexibility*: the configuration and specification of the instrument can be modified and adapted at any time to achieve your specific scientific progression, and it offers *customization*: a broad range of bio stimulation features dedicated to your bioprinting process. Moreover, Marc Thurner concludes: “It is the unique bioprinting solution to discover the undiscovered!”

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CELLINK – Accessible to Everyone

The Sweden-based biotech company develops novel, cell-specific bio-inks for 3D bioprinting of human tissues, such as cartilage, skin and bone. Their benchtop 3D bioprinters are aimed at scientists who want to try out 3D bioprinting and start bioprinting living tissues. The tissue models bioprinted with CELLINK technology are used for 3D culture of human-like tissues, development of in vitro toxicology and cancer models, among other applications. CELLINK created headlines with BIO-X, a device that puts researchers at the cutting edge by being the first bioprinter world-wide with intelligent printheads. BIO-X printheads are characterized by flexibility, beauty and simplicity. “Even if you design your own dispensing technologies, you can use them with the BIO-X system”, says **Dr Héctor Martínez**, co-founder and CTO of CELLINK, underlining the benefits. With the triple printhead technology of BIO-X you can combine different materials and printing techniques to fit your application. You are free to choose from up to 7 different printheads, including a 250°C pneumatic head, a cooled pneumatic head, an ink-jet printhead, a syringe pump printhead and an HD camera tool head. “The possibilities are limitless and give users full freedom to expand their bioprinting research and applications”, confirms Dr Héctor Martínez. “BIO-X is the easiest-to-use bioprinter on the market and a complete standalone product.”



CELLINK's BIO-X, the most user-friendly 3D bioprinter on the market. An advantage is the easy-to-use software, designed to simplify the bioprinting process by guiding the user along every step of the way. Source: CELLINK.

One of CELLINK's ultimate achievements is bringing robust and easy-to-use bioprinters to the hands of researchers at an affordable price. Watch the INKREDIBLE+ 3D bioprinter print human tissue right in front of users' eyes. The instrument is surprisingly accurate, features a high reproducibility rate and is easy-to-use. “The INKREDIBLE+ 3D bioprinter has been optimized to print soft tissues with human cells embedded in a bioink matrix, however, the sky is the limit as to what tissues you can print”, says Dr Héctor Martínez and adds with a smile: “The world is waiting to see what you will print next!”

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Where is this taking us?

As **Professor Michael Raghunath** explains, bioprinting started off when cells in solution were delivered through inkjet printer heads: “What started as a crazy idea has become a popular cutting-edge tissue engineering technology. As the basic technical problems in controlling the actual 3D liquid dispensing and structuring appear to be basically solved, other challenges move into the foreground.” The medium the cells are printed in, the so-called bio-ink, is a challenge to compose as it should protect cells against shear forces during the printing process but also be viscous enough to keep some shape fidelity after printing and before crosslinking. “There is still some work to be done to improve current bio-inks to make them more cell-friendly”, states the scientist. “Many bio-inks are good for printing, but do not contain enough biochemical cues for cells to engage the printed scaffolds. To maintain structural integrity the printed bio-ink structures need to be chemically crosslinked, mostly by using UV and visible light.” The big question, highlighted by the Head of Cell Biology and Tissue Engineering, was: what happens after printing? Will the bioprinted cells be able to build their own microenvironments and to remodel them while gradually replacing the original bio-ink scaffold? As the researcher points out, most if not all bio-inks are hydrogels, *i.e.* structures composed of entangled polymers that can hold considerable amounts of water. He therefore suggests that “where there is water there is the option to apply macromolecular crowding”, a technology he has developed that drives cells in culture to build their own microenvironment (extracellular matrix) much faster than in standard aqueous uncrowded culture conditions. And he concludes: “Perhaps this might help us to achieve a better post-printing maturation of tissue. Obviously, many interesting and exciting developments lie ahead in the field of 3D bioprinting.”

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