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biotechnet switzerland

SWISS BIOTECH National Thematic Network

# Conference Report

## Olten Meeting 2015

## Antibiotics and Bioprinting – for a better life

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*Abstract:* The ever lurking danger of antibiotic resistance and the potential of bioprinting are on everyone's lips. But where do we stand in the battle against antibiotic-resistant pathogens? And what are the opportunities for biotech in the 3D printing of biological tissues and organs through the layering of living cells? At the Olten Meeting 2015 scientists and entrepreneurs met to throw light on the current situation.

**Keywords:** Olten Meeting 2015 · Swiss Biotechnet

Biotechnology is the manipulation and application of living organisms or their components to produce useful commercial products such as pharmaceuticals, diagnostics, resistant crops, tissues and biomaterials. Over the last 20 years, biotechnology has dramatically broadened in terms of its scope and sophistication, hence also in its applicability. "Various industry sectors have integrated biotechnology in their value chains. Biotechnology is one of the core technologies in the pharma value chain, providing data and results from early drug discovery through to late clinical development", explains Dr. ***Daniel Gygax***, President of biotechnet and Professor at the School of Life Sciences FHNW Muttenz. "The National Thematic Network Swiss Biotech bridges activities in industry and academia to increase competitiveness by mediating core competencies of participating companies with academic knowledge and practices. This is efficiently done by using a set of instruments: events, workshops, the NTN bulletin, reports and publications and the NTN Swiss Biotech competence platforms." Two of the six platforms were addressed at the Olten Meeting 2015, namely antibiotics and tissue engineering.

All over the world researchers work to improve antibiotic effectiveness. The range of activities is very broad, from new drugs offering relief to patients to hospitals fighting antibiotic resistance.

### Pooling Expertise on a National Platform

Introducing the topic of antibiotic resistance, ***Markus Seeger***, Professor at the Institute of Medical Microbiology, University of Zurich, gave a brief report on the state of play regarding the creation of the first of the NTN Swiss Biotech Platforms concerned with Antibiotics, of which he is the head (see CHIMIA **2015**, *69*, 809). The goal is to push promising technologies by bringing together specialists in antibiotics research from academia and industry to exploit existing synergies and generate quantifiable results more rapidly. "The spread of antibiotic resistance worldwide is frightening", comments Markus Seeger. "While we think that we have Gram-positive pathogens under control, the alarming increase in antibiotic-resistant Gram-negative infections forces us to act quickly." Gram-negative bacteria are generally much less permeable to antibiotics and thus less susceptible to naturally occurring antibiotics – a real challenge for the international research community.

### How Bacteria Pump out Antibiotics

Many bacteria, such as *Escherichia coli* or *Pseudomonas aeruginosa,* possess a pump that is able to eject harmful substances out of the bacterial cell in a highly efficient way. By increasing the expression of efflux pumps in the presence of antibiotics, the impact of antibiotics is reduced, thereby ensuring the survival of bacteria. One drug efflux pump can extrude myriads of drug molecules, leading to multi-drug resistance (MDR).

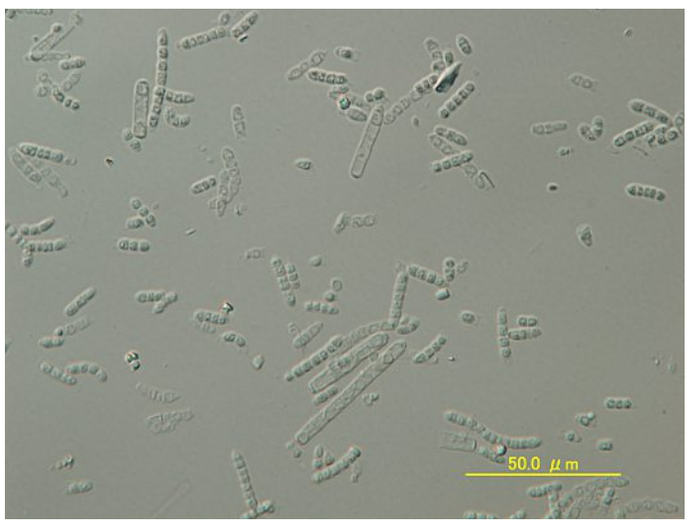
At the very forefront of research in this domain is Professor Markus Seeger, research group leader at the Institute of Medical Microbiology of the University of Zurich and Head of the Antibiotics Platform of biotechnet. "We examine, for instance, tripartite efflux pumps like the Acr/AcrB/TolC protein complex from *Escherichia coli,* which are major contributors to the drug-resistance pump in Gram-negative ESKAPE pathogens", explains the scientist. "More recently we elucidated the structure of an ABC transporter that pumps antibiotics out of the cell at the expense of ATP hydrolysis. ABC transporters mediate intrinsic antibiotic resistance mainly in Gram-positive cells." In order to understand the molecular details of the pumping mechanisms that lead to antibiotic efflux, he uses X-ray crystallography, antibiotic binding and transport studies and mutational analysis of residues lining the antibiotic efflux tunnel. Crystal structures determined by Professor Seeger and his group feature tunnels inside the protein and imply a 'peristaltic mode' of drug transport that could account for the wide substrate specificity observed. "Drug efflux pumps are part of the intrinsic resistance of bacteria towards drugs", Markus Seeger sums up. "There is a promising strategy to treat bacterial infections: by inhibiting drug efflux pumps the bacterial cell becomes more sensitive towards an entire set of antibiotics."

*www.imm.uzh.ch*

### Microbial Dark Matter: A Rich Drug Discovery Resource

In his research ***Jörn Piel***, Professor at ETH Zurich and Lab leader at the Institute of Microbiology, focuses on natural products from symbionts of marine animals. Scientists in his group use biosynthetic knowledge to generate structural diversity not yet encountered in nature to create a sustainable source of rare bioactive compounds. Such bioactive natural products from bacteria are also the basis of numerous antibiotics. However, these compounds are derived from only a minute portion of the entire spectrum of bacterial diversity, since most bacteria have so far resisted cultivation. "Many uncultivated bacteria belong to large taxonomic groups with no cultivated representatives or known functional properties," comments the researcher. "It has been proposed that these elusive organisms represent a massive untapped resource of novel drug candidates and biotechnologically useful enzymes." To investigate the biosynthetic potential of this *microbial dark matter*, he uses meta-genomic and single-cell methods focusing on host-associated microbiomes. "We provide evidence for the existence of chemically *talented* environmental producer groups that are metabolically highly versatile", he explains. "These bacteria exhibit a rich specialized metabolism with bioactive compounds not encountered in microbes that are

typically studied in drug discovery programs." To access these metabolites, his research group is pursuing several avenues, including the development of cultivation techniques, heterologous gene expression, and identification of alternative producers by genome mining.



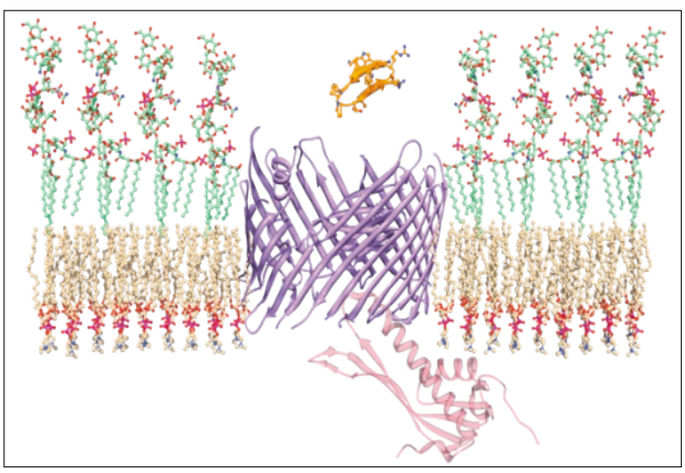

Institute Microbiology/ETHZ: Many microbial symbionts of marine sponges are highly host specific and cannot be cultivated outside of their host environment. This symbiont "*Candidatus Entotheonella* factor" produces many bioactive substances in the sponge *Theonella swinhoei.* Photo Tetsushi Mori.

*www.micro.biol.ethz.ch/research/piel*

## ESKAPE – The 'Escaping' Pathogens

Dr. ***Francesca Bernardini***, Project Manager Antibiotics and Group Leader Microbiology at Polyphor Ltd. in Allschwil, looks behind the scenes of this company, which focuses on the discovery and development of macrocyclic drugs. These constitute a novel class of drugs (MW: ~400–2'000 Da) located in terms of size between the well-established small molecules and large biopharmaceuticals. Like large biopharmaceuticals, Polyphor macrocycles can address complex biological targets, while also featuring certain advantages of small molecules, such as good bioavailability and tissue penetration. Polyphor, a pioneer in the field, has established a proprietary macrocycle platform consisting of the PEMfinder® and MacroFinder® technologies. PEMfinder® is based on conformationally constrained cyclopeptides (MW: 700–2'000 Da) mimicking beta-hairpin and alpha-helical epitopes in proteins involved in protein-protein interactions.[1] MacroFinder® is based on non-peptide-based scaffolds (MW: 400–800 Da) that are cell-permeable and can be made orally bioavailable. Hence, PEMfinder® and MacroFinder® are highly complementary in addressing extra- and intracellular complex biological targets.

"Polyphor applies its macrocycle platform for both internal and external drug discovery and development projects, partly in collaborations with Novartis, Boehringer Ingelheim, and Takeda", explains Francesca Bernardini. "Our platform is extremely versatile and can be applied in many therapeutic areas. We focus on therapeutic areas of high medical need where macrocycle drugs have the potential for providing significant clinical benefit." Polyphor currently has three clinical-stage products derived from PEMfinder® in its portfolio: POL7080 (Phase II), a highly selective antibiotic with a novel mode of action for treating Pseudomonas infections; POL6326, Balixafortide, (Phase II), a CXCR4 antagonist, for tissue repair and combination treatments in oncology; and POL6014 (Phase I), an inhaled inhibitor of neutrophil elastase for the treatment of alpha-1 antitrypsin deficiency, cystic fibrosis, and other lung diseases. However, Francesca Bernardini warns: "Due to the rapid emergence of multidrug-resistant (MDR) pathogens and the absence of innovative drugs being developed, an antibiotic crisis is spreading, placing a considerable strain on our health economic system.[2] We see a high medical need for novel antibiotics with previously unexploited mechanisms of action (MoA) that are not affected by existing resistance mechanisms. In a series of alarming reports the Infectious Diseases Society of America (IDSA) has identified so-called ESKAPE pathogens, against which our current arsenal of antibiotics is becoming increasingly ineffective. The IDSA has launched the 10 X 20 initiative calling for ten new antibiotics to be developed by 2020."



Polyphor: Protein Epitope Mimetics (PEM) antibiotics in action. Photo by Polyphor.

Meanwhile, Polyphor has successfully applied its macrocycle platform to discover novel broad- and narrow-spectrum antibiotics against Gram-negative ESKAPE pathogens, including MDR-strains. This step was successful: Starting from the antimicrobial host defence peptide protegrin I, Polyphor and Prof. John Robinson at the University of Zurich have jointly discovered a novel class of highly potent macrocycle antibiotics with a novel MoA that is selectively active against *Pseudomonas aeruginosa*.[3] Optimization of initial hits eventually lead to the clinical candidate POL7080. This compound inhibits the outer-membrane transporter LptD and interferes with LPS export and outer-membrane biogenesis and is currently in Phase II clinical development in VAP (ventilator-associated pneumonia). "Leveraging the experience and the knowledge acquired in the course of the discovery and development of POL7080, Polyphor is developing a novel series of macrocycle antibiotics with broad-spectrum activity against Gram-negative ESKAPE pathogens, including MDR- and colistin-resistant strains", summarizes Francesca Bernardini. "Colistin is considered as a last resort antibiotic against life-threatening Gram-negative infections, although colistin-resistance is rapidly emerging."

*www.polyphor.com*

[1] D. Obrecht, E. Chevalier, K. Moehle, J. A. Robinson, 'β-Hairpin protein epitope mimetic technology in drug discovery', *Drug Disc. Today: Technologies* **2012**, *9*, e63–e69.

[2] D. Obrecht, F. Bernardini, G. Dale, K. Dembowsky, 'Emerging New Therapeutics Against Key Gram-Negative Pathogens', *Ann. Rep. Med. Chem.* **2011**, *46*, 245–262.

[3] N. Srinivas, P. Jetter, B. J. Ueberbacher, M. Werneburg, K. Zerbe, J. Steinmann, B. Van der Meijden, F. Bernardini, A. Lederer, R. L. Dias, P. E. Misson, H. Henze, J. Zumbrunn, F. O. Gombert, D. Obrecht, P. Hunziker, S. Schauer, U. Ziegler, A. Käch, L. Eberl, K. Riedel, S. J. DeMarco, J. A. Robinson, 'Peptidomimetic Antibiotics Target Outer-Membrane Biogenesis in *Pseudomonas aeruginosa*', *Science* **2010**, *327*, 1010–1013.

## Bioprinting – What is Really Involved

A well-known specialist in the domain of bioprinting is Dr. ***Fabien Guillemot***, founder and CEO of Poietis. This bioprinting company harnesses the Laser-Assisted Bioprinting technology to fabricate complex and customized tissues for regenerative medicine and pharmaceutical applications. It is located in the heart of Bioparc Bordeaux Métropole, the technology business park linking science and technology, with the nearby university hospital centre of Bordeaux. Fabien Guillemot and his team deal with tissue complexity in the knowledge that reproducing the functional anisotropy of human tissues remains a puzzling challenge for tissue engineers. Emergence of the biological functions results from dynamic interactions between cells and with the extracellular matrix. "The important literature showing that cell fate (migration, polarization, proliferation…) is triggered by biochemical and mechanical signals arising from the cell microenvironment suggests that tissue formation obeys short-range orders without reference to a global pattern", explains Fabien Guillemot. "In this context, the winning tissue engineering strategy might rely on controlling tissue organization at the cell level."

Emerging during the last decade, bioprinting has been defined as "the use of computer-aided transfer processes for patterning and assembling living and non-living materials with a prescribed 2D or 3D organization in order to produce bio-engineered structures for use in regenerative medicine, pharmacokinetic and basic cell biology studies". From a technological point of view, the Laser-Assisted Bioprinting (LAB) technology has been developed as an alternative method to inkjet and bioextrusion methods, thereby overcoming some of their limitations (namely clogging of print heads or capillaries) to pattern living cells and biomaterials with a micron-scale resolution.

"By harnessing this high printing resolution, we observe that tissue self-organization over time depends on the cell patterns initially printed by LAB, as well as cell types", concludes Fabien Guillemot. "To engineer complex tissues, we then emphasize the need to consider the spatio-temporal dynamics of tissue self-organization when designing blueprints."

*www.poietis.com*

Poietis: Laser-Assisted Bioprinting workstation developed by INSERM at the University of Bordeaux. Photo by INSERM

## Bio-INK, Development for the Printing of Osteochondral Grafts

As a PhD student at the Department of Health Sciences and Technologies at ETH Zurich, ***Mischa Mueller*** is interested in cartilage engineering and regeneration, especially the bio-ink development for the printing of osteochondral grafts. Osteoarthritis is a common disease causing joint pain and functional disability. With an aging and more active population, the number of people suffering from osteoarthritis will most likely increase in the future, yet current therapies still fail to deliver satisfactory results. "The repair of such defects requires the creation of so-called ostechondral grafts, which consist of two or more distinct layers that account for the different repair needs of the injured bone and cartilage tissue", explains Mischa Mueller. "3D printing is a layer-by-layer approach that could be used in the future to create such osteochondral grafts, tailored to the defect geometry of each individual patient. However, materials for the 3D printing of such grafts are scarce, and their development is crucial for the progression of this technology in the tissue engineering field." The scientist is examining the current challenges in the material development of inks for osteochondral grafts and drafting a vision showing how 3D printing could be used to learn more about the formation of cartilage and bone. The goal is to achieve a better understanding of how to repair osteochondral lesions.

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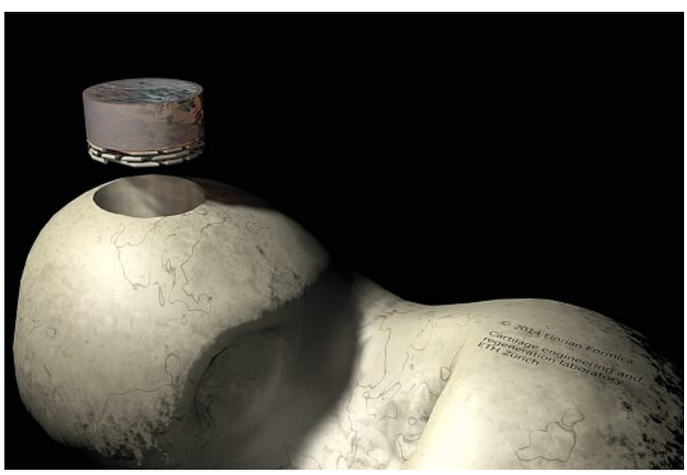
## Switzerland – A Step Ahead

For years the Institute of Chemistry and Biological Chemistry, ZHAW Waedenswil, has been cooperating closely with the biomedical company regenHU Ltd. and has accumulated considerable expertise in this area, with the result that Switzerland is now one of the world-leaders in bioprinting. Accordingly, Dr. ***Markus Rimann***, senior scientist, is reviewing the state of the art in this domain. Today, three-dimensional (3D) cell cultures are well established. Standard tissue engineering approaches generate 3D models with random distribution of cells and matrices,



HEST/ETHZ: Vision for 3D-printed osteochondral grafts in the future. Photo by Florian Formica, ETHZ.

but this does not reflect the *in vivo* situation. "There is an urgent need for standardized and reliable *in vitro* 3D models for substance testing in the cosmetics and pharma industries", says Markus Rimann. "Bioprinting that allows the precise deposition of cells, matrices and biological factors in 3D is expected to generate advanced *in vitro* tissue models in a reliable manner that better reflects the *in vivo* situation."

He and his group establish robust protocols for printing soft tissue models in a reproducible way. The bioprinter is equipped with micro valve-based inkjet print heads for cell jetting and contact printing. A chemically-defined ECM-like BioInk that is print- and cyto-compatible has been developed. For the light-induced polymerization a UV-LED (365 nm) was integrated in the instrument. Tissues are printed as follows: First, one layer of BioInk is printed and polymerized with UV and then cells suspended in cell culture media are jetted in the same pattern onto the BioInk, followed by the next BioInk layer. In this way, the 3D tissue is printed.

In a proof-of-concept study the ZHAW team printed full-thickness skin equivalents. Dermal equivalents were printed with eight layers of human primary dermal fibroblasts. "Viability stainings (MTT) showed proliferating cells throughout the whole culture period of 7 weeks, and cells were populating the entire constructs", explains Markus Rimann. "In different time points, human primary keratinocytes were seeded on top of the dermis, leading to an epidermal-like layer as shown by immunostaining. Bioprinting could provide customized skin models for the cosmetics industry to test the influence of cosmetic ingredients."

In an ongoing project the ZHAW researchers are developing *in vitro* muscle/tendon tissues in a customized labware allowing read-out in the same well plate. The specialized 24-well plate contains two posts in each well to produce muscle fibres between the posts. "With the previously developed BioInk we printed primary human myoblasts in a dumbbell-shape that differentiated into striated myotubes as shown with myosin heavy chain (MHC) staining", comments Markus Rimann. "Printed primary rat tenocytes showed characteristic collagen I distribution around the cell nuclei after differentiation. In the future, bioprinted muscle/tendon tissues could be used in compound screening for muscle-related diseases."

With the developed bioprinter the ZHAW is able to produce *in vitro* skin and muscle/tendon tissue models with primary cells combined with customized labware for read-outs.

*www.icbc.zhaw.ch*

Information on NTN Swiss Biotechnet: *www.biotechnet.ch*

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