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Hot from the press!

Conference Report 2016 TEDD Annual Meeting, 27. October, 2016, ZHAW Wädenswil

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Abstract: The focus of the 2016 TEDD Annual Meeting – the Competence Centre for Tissue Engineering for Drug Development and Substance Testing – was on current segments of the 3D cell culture market, especially scaffold-free and scaffold-based cell culture, as well as bioprinting, and the use of recellularized tissues. Particular emphasis was placed on metabolic tissue engineering, specifically the generation of human brown fat cells from progenitor cells. Let's take a look behind the scenes of latest developments.

Keywords: Bioprinting · 3D Cell culture technology · Engineering human hair follicles · Human brown fat cells · PhytoCellTec™ method · Recellularized tissue · Spheroid-based microphysiological system

Originally, brown fat was assumed to be present only in babies and small infants, but there is increasing evidence to suggest that adults also have brown fat deposits in varying degrees. “What makes brown fat so interesting is its capacity to become a fuel guzzler after activation, increasing the metabolic rate and burning calories”, notes Professor Michael Raghunath, the new director of TEDD, who assumed the leadership of the Centre for Cell Biology and Tissue Engineering at ZHAW's Life Sciences and Facility Management in summer 2016. He chose this topic to present new technologies that empower cells to build their own microenvironments and thus perform at a higher physiological level. This annual TEDD meeting also conveyed information about current activities in research and industry in this field.

Regulation of Brown Fat Formation and Function

While obesity is defined as an expansion of adipose tissue mass, in recent years it has become evident that not only the mass, but also the quality of adipose tissue is important. “Adipose tissue can be subdivided into two distinct types, namely white and brown fat. White fat is specialized in the storage of lipids. However, under obese conditions it is transformed into inflamed tissue, which contributes to the development of obesity-associated co-morbidities. Brown fat, by contrast, releases energy in the form of heat through uncoupling, leading to an enhanced basal energy expenditure”, says Christian Wolfrum, Associate Professor of the Institute of Food Nutrition and Health at ETH Zurich. Brown adipose tissue has attracted considerable interest in recent years as a target organ for improving metabolism. At the moment there is controversy surrounding the mechanism by which brown adipocytes are formed and become activated in response to external cues. “It is well established that brown fat cells can develop from adipocyte precursors”, states the chemist,

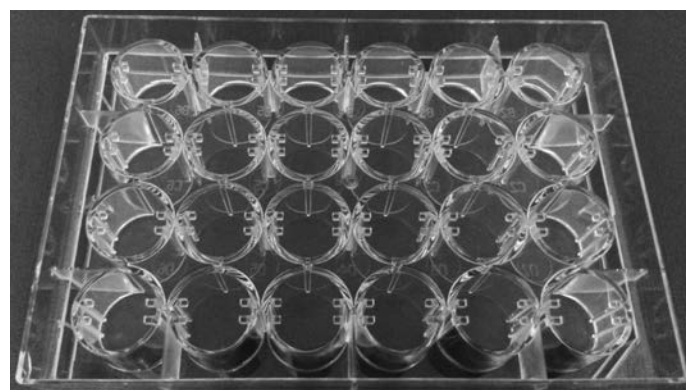
who spent six years at the Rockefeller University in New York, USA. “Nevertheless, a large body of evidence suggests that brown adipocytes can acquire a ‘white adipocyte like phenotype’ under un-stimulated conditions, suggesting a certain degree of plasticity within the adipose tissue organ.

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Fabrication of Functional Human Skeletal Muscle Tissue for Drug Testing Using 3D Bioprinting

Today, international markets are putting pressure on global pharmaceutical companies like Novartis. In order to keep pace, the pharmaceutical giant in Basel focuses on innovation, as the development of a new product – from the initial idea to the market lunch – takes 10 to 16 years and consumes about 2.5 billion CHF. “In the search for new active ingredients, we expect to use a great deal of engineered 3D functional human tissues”, says Hansjoerg Keller, Senior Investigator at Novartis Pharma AG in the Musculoskeletal Disease Area. In a project with the TEDD crew at ZHAW he is developing functional human skeletal muscle tissue for drug testing with this method.

Degenerative skeletal muscle diseases are creating a growing medical burden in our aging societies. Furthermore, muscle wasting is the cause of fatal diseases such as Duchenne muscular dystrophy and amyotrophic lateral sclerosis, which affect both children and adults. Currently, there are no effective medicines for these illnesses. A major hurdle for efficient drug discovery is the lack of functional *in vitro* tissue assays for compound screening. “With 3D printing we have a revolutionary new technology for the fast production of complex, three-dimensional objects by computer-aided design (CAD) or 3D scanning. 3D bioprinting and its application to life sciences is also emerging as a new technique to revolutionize the engineering of 3D living tissues for applications in drug development and regenerative medicine. Ultimate goals are the production of functional organs, or parts of organs, for replacement surgery. Although this is, at best, still years away from reality for complex organs such as the kidney or heart, significant advances have been reported for *in vitro* engineering of 3D functional tissues using 3D bioprinting”.



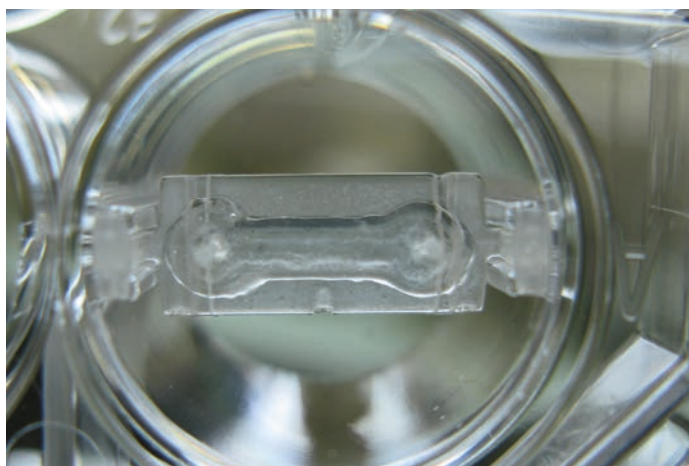
Top view of the novel 24-well plate for postholder inserts. Source Novartis Basel.



Single well with inserted postholder insert. Source Novartis Basel.

explains Hansjoerg Keller, who has many years of experience in cell biology, endocrinology and molecular biology. The scientist has employed 3D bioprinting to generate functional 3D skeletal muscle fibre tissues *in vitro* from precursor cells for the testing of drug candidates. To this end, his group has developed a novel multiwell tissue culture plate system that contains cell culture inserts with two posts for muscle fibre attachment. Tissues were fabricated by printing muscle precursor cells in a dumbbell shape around the two posts on the cell culture inserts.

“To hold printed cells in space and build 3D structures, we have used different ‘bioinks’, including PEG-based hydrogels and Matrigel using different printing modes”, the Novartis researcher recalls. “Bioink and cells were printed in alternating layers, whereas Matrigel and cells were printed together. Cells were >90% viable after printing and proliferated well in growth medium. After switching to a differentiation medium, myoblasts fused to form multinucleated myotubes, as shown by specific myosin heavy chain immunostaining and qPCR marker gene expression analysis. Furthermore, cross-striation of myotubes indicated sarcomeric structures, which are the basis for muscle fibre contractions. Finally, spontaneous and electrically-



3D bioprinting of Bioink on postholder inserts. Source Novartis Basel.



3D bioprinted human skeletal muscle cell tissue spanning between posts 1 day after printing. Source Novartis Basel.

induced contractions were observed in mature muscle fibre cultures.” In summary, Hansjoerg Keller and his project partners presented the generation of functional skeletal muscle tissues on novel two post-containing multiwell cell culture inserts using 3D bioprinting. These *in vitro* human tissue assays will be employed in compound screenings for the development of novel drugs against muscle-wasting diseases.

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Pioneering Work in Plant Stem Cell Technology

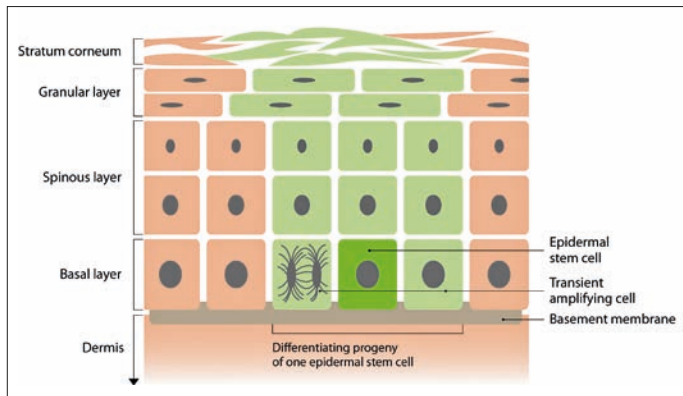
The Swiss company Mibelle Biochemistry – an independent business unit of the Mibelle group – was founded in 1991 by Dr. Fred Zuelli. It develops unique and high-quality active components based on natural ingredients and scientific research for the cosmetic industry. Today a broad range of active substances is available in more than 50 countries around the globe. In his talk Fred Zuelli presented the biotechnology PhytoCellTec™, introduced in 2008, for the generation and cultivation of plant stem cells.

As Fred Zuelli explains, the skin undergoes constant cell turnover in order to maintain, renew and repair its tissue. Responsible for this regenerative capacity are adult stem cells residing in special niches in different layers of the skin. Stem cells are defined by their ability to self-renew and to differentiate into mature specialized cell types. However, stem cells are also subject to aging, resulting in reduced viability and a decreased stem cell pool. Since the depletion of stem cells is a major cause of skin aging, cosmetic ingredients that vitalize skin stem cells have a real anti-aging potential. This is a novel story for the cosmetic industry. As Fred Zuelli continues, Mibelle Biochemistry has



With PhytoCellTec™ the growth of callus cells (wound tissue) in selected plant tissue is induced under special conditions. These callus cells are undifferentiated plant cells, in other words, stem cells. Source Mibelle Biochemistry.

developed a novel plant cell culture technology, PhytoCellTec™ based on the unique totipotency of plant cells. “An extract of apple stem cells was tested for anti-aging effects on skin. For this purpose a human keratinocyte progenitor cell line was established as *in vitro* test system. The activity of these epidermal stem cells can be assessed based on their characteristic property of growing in colonies. Stem cell cultures incubated with the apple



PhytoCellTec™, *Malus Domestica*, a preparation of apple stem cells, was found to stimulate the proliferation of human blood stem cells and to increase the lifespan of skin fibroblast cells and of isolated hair follicles. Source Mibelle Biochemistry.

stem cell extract form almost twice as many colonies compared to the untreated control culture.” This clearly indicates that the apple stem cell extract helps epidermal stem cells maintain their characteristic function. The activity of plant stem cells on human skin stem cells with the additional use of 2D and 3D cell culture techniques was demonstrated.

“Starting from these initial experiments with apple stem cells the cosmetic industry has generated a new global trend, ‘Stem Cell Cosmetics’”, explains Fred Zuelli, Managing Director Biochemistry. “The use of plant-derived stem cells in all types of cosmetic formulations facilitates the protection and vitalization of human skin stem cells. This offers novel anti-aging concepts. Hundreds of brands around the world have launched new products based on this concept since 2008, when apple stem cells were first offered to the market.”

www.mibellebiochemistry.com

2016 TEDD Annual Meeting

As Michael Raghunath points out, the role of the extracellular matrix (ECM) as a microenvironment of differentiated cells and stem cells is increasingly acknowledged as an important biological driver of stemness and differentiation. “In a bid to build better ECM microenvironments we have developed macromolecular crowding (MMC) as a biophysical principle for tissue engineering. Macromolecules of defined hydro-dynamic radius added to cell culture media modulate ECM formation in several ways”, recalls the internationally renowned clinician-scientist specialized in matrix biology and skin biology. “Firstly, MMC enhances the conversion of procollagen to collagen, a prerequisite for supramolecular assembly. Secondly, MMC tunes collagen fibrillogenesis and architecture. Thirdly, it stabilizes ECM by increased enzymatic crosslinking and its remodelling.” Although many macromolecules could potentially serve as crowders, stable and neutrally charged macromolecules are preferred, for example sucrose copolymer and polyvinylpyrrolidone. The combination of neutral crowders of different sizes – mixed macromolecular crowding – is particularly efficacious both in simple culture systems and organotypic constructs, and can be combined with cell sheet technology.

Recently, MMC (macromolecular crowding) exclusively facilitated the differentiation of human mesenchymal stromal progenitors into brown adipocytes. “We discovered that, in contrast to standard culture, MMC facilitates the formation of an all-surrounding cocoon of collagen IV, which leads to the formation of focal adhesions also on top of differentiated adipocytes”, says the scientist, who has a strong background with over 25 years’ experience in academic, clinical and industrial research. “Thus, MMC induces a novel spatiotemporal ECM (extracellular matrix) engagement and signalling in monolayer culture, giving a new twist to the term “3D” in cell culture. It is now planned at ZHAW to exploit this finding to build a nutraceutical and pharmaceutical screening platform for the activation of human brown fat cells or the browning of white fat cells to assume the functionality of brown fat cells.”

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Received November 19, 2016