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**Hot from the press!**

# TEDD & 3R Workshop at ZHAW Waedenswil on 7 April 2017 Organized in collaboration with Animal Research

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*Abstract:* On April 7, 2017, TEDD (Tissue Engineering for Drug Development and Substance Testing) and the Animal Research Foundation joined forces at ZHAW Waedenswil to present their latest scientific results in the domain of the 3Rs of biomedical research: how to Refine, Reduce and Replace animal testing with alternative approaches.

**Keywords**: Animal-free research · Fetal calf/bovine serum · Full-thickness skin models · Human platelet lysates hPL · Rabbit silicon ear · Human skeletal muscle and tendon tissues · Three-dimensional bioprinting

One promising avenue towards animal-free research is the use of sophisticated *in vitro* cell and tissue models using human cells. The use of animal products is still widespread in cell cultivation, namely Fetal Calf Serum (FCS). FCS or FBS (Fetal Bovine Serum) – as it is also called today – is the blood fraction remaining after the coagulation of blood, followed by centrifugation to remove any remaining red blood cells. Today it is an essential admixture to animal and human cell culture media as it has a very low level of antibodies and contains growth factors, allowing for versatility in different cell culture applications. But Dr. ***Gerhard Gstraunthaler***, from the Division of Physiology at Medical University of Innsbruck, lists a series of disadvantages.

TEDD and Animalfree Research discuss the challenges of serum-free cell culture on the occasion of the TEDD & 3R Workshop organized at the ZHAW Waedenswil on 7 April 2017. © ZHAW Waedenswil.

## Quo vadis FBS?

"These include unknown composition, seasonal and geographical lot-to-lot variability, safety concerns in terms of endotoxins, mycoplasma, viral contaminants or prion proteins and unpredictable shortages in global supply", says the professor. "Most serious are the ethical concerns about animal welfare and the collection of blood from unborn bovine fetuses. This is a cause of concern, as recent figures estimate that approx. 800,000 liters of FBS are produced annually worldwide, corresponding to the harvesting of 2,000,000 bovine fetuses, and the numbers continue to grow." The fact remains that the global supply of FBS is still a dark market that is only loosely regulated: exact numbers for both global production of FBS and availability, as well as the worldwide demand are still unknown. This situation could create opportunities for abuse and fraudulent blending of FBS. "In 1994, it was reported that approx. 30,000 liters of 'New Zealand' serum were sold worldwide. However, only 15,000 liters of high-quality FBS were collected annually in New Zealand."

## A solution for the future?

The search for strategies to reduce or replace FBS in cell culture is experiencing a boom. The recently developed activated human donor platelets (human platelet lysates, hPL) appear very promising. "The rationale behind the use of hPL is the clotting process itself, which includes the activation of platelets and the exocytic release of α-granule factors", explains the researcher. "Platelet α-granules contain an array of growth factors required for wound healing and tissue regeneration, but they have also been identified as being essential for cell attachment, growth and proliferation *in vitro*." The conditions look good: "hPL are obtained from expired human donor platelets, a recognized, ethically approved, safe and clinically tested, high-quality product. With hPL, bulk platelet growth factors are added to basal culture media, providing a human-based, xeno-free culture system."


Professor Gerhard Gstraunthaler from the University of Innsbruck is critical as to the widespread use today of Fetal Bovine Serum and presents options as alternatives to FBS. © ZHAW Waedenswil.

As Gerhard Gstraunthaler and his team proved, the cocktail of platelet factors supported growth and proliferation of human and animal cell lines. The addition of hPL to quiescent culture stimulated the ERK1/2 MAPK signaling pathway, strongly indicating the mitogenic potential of platelet α-granule growth factors. "We showed that adipose-derived mesenchymal stem cells (ADSC) could be expanded under hPL-supplemented culture conditions, and that the cells maintained their undifferentiated oligopotent phenotype," explains the Austrian expert. "In addition, ADSC under hPL were able to retain their full mesodermal differentiation potential towards adipogenic, osteogenic and chondrogenic phenotypes. That means hPL is the most valuable replacement of FBS in the culture of animal and human cells, including adult stem cells."

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## Improving Reproducibility in Science

Scientific progress is assured by trusted discoveries, recalls Professor ***Thorsten Buch***, Head of the Institute of Laboratory Animal Science (LTK) at the University of Zurich. As well as conducting research, every year the LTK introduces hundreds of Swiss and international scientists to the appropriate methodology for conducting human and scientifically sound animal experiments.

The Transgenic Technologies Laboratory at the Institute of Laboratory Animal Sciences (LTK) gives scientists access to state-of-the-art technology for their research. Picture: Institute of Laboratory Animal Sciences LTK

To gain confidence in their conclusions, it is important for them to reproduce experiments. "Most scientific results are not reproducible," states the biologist, citing headline news articles from scientific journals and newspapers. "But reproducibility enables the scientist to support an experimental hypothesis and to turn it into accepted knowledge." Science works on the assumption that what we discover today will also be true tomorrow. It thus facilitates prediction. "For animal experiments the reproduction of results has been called into question due to the apparent, or imagined, lack of translation into clinical – human – application", comments Thorsten Buch. "Reproducibility has been associated with measures of good scientific practice and claims to the effect that absence of these actions implies absence of reproducibility." Such measures usually include standards of statistical and experimental planning.

But are these measures truly indicators of reproducibility? Is the sloppy use of such standards the cause of the apparent reproducibility crisis, or do other factors contribute to the variability seen in the outcomes of experiments in biomedical research? What causes of irreproducibility can be avoided, and what are elementary features when it comes to gathering new scientific knowledge? Thorsten Buch suggests ten points to improve biomedical science – from training through lab organization to publication standards. At present, science journals insist that each paper contains a story, and this story should not be descriptive but should reveal the underlying mechanisms. "But let's be realistic: We do not need top-selling stories about scientific results, but the description of observations – it's the only thing that counts!" To support such a shift, the LTK-run animal welfare website Swiss3Rnetwork has entered into a collaboration with the new online journal *Science Matters*, based in Zurich. Thorsten Buch closes by suggesting that, when working with animals, researchers should add three more 'Rs' to the existing ones: Respect, Responsibility, Reproducibility.

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## 3R – An Important Factor in Pharmaceutical Research

The fact that alternatives to animal testing should more closely reflect human biology and result in a better prediction of efficacy and safety of new medicines may explain the appearance of animal-free research in industrial labs. As Dr. ***Birgit Ledermann*** explains, the implementation of the 3Rs in animal research is a high priority for Novartis. The multinational pharmaceutical company, where she is responsible for the Novartis 3Rs programme, fully supports the use of alternatives to animal research wherever feasible. "Today, the 3Rs are increasingly important, with a greater focus on developing alternatives to animal testing," says Birgit Ledermann, who also serves as president of the Swiss Laboratory Animal Science Association. Since 1987 this organization has served as an intermediary between animal welfare and research for the benefit of humans and animals, and is a key partner for authorities, scientists and public, promoting the 3Rs. "Scientists at Novartis are urged to search for alternative ways to conduct studies before undertaking animal research," she comments. "To help achieve this aim, Novartis adheres to the principles of the 3Rs. Their implementation is applicable to a variety of areas, including the environment, nutrition, health status of the animals, procedural training, education and, finally, animal experimentation."

As an example of education and training at Novartis, she mentions *the rabbit silicone ear* invented by a Novartis animal caretaker. This is a patented training device for taking blood and carrying out intravenous injections in rabbit ear veins and arteries. In this way it replaces the need to use live animals in initial basic exercises for trainees in animal experimental techniques.

Novartis adheres to the principles of the 3Rs, implemented in a variety of areas, including the environment, nutrition, health status of the animals, procedural training, education and, finally, animal experimentation. Copyright Novartis.

A further example of replacement concerns a close collaboration between Novartis and the TEDD group at ZHAW Waedenswil. The aim is to replace animal testing in musculoskeletal disease areas such as sarcopenia and cachexia. In collaboration with TEDD, Novartis has developed a methodology for engineering human skeletal muscle and tendon tissues using 3D bioprinting. Since Novartis believes in the future reduction, refinement and replacement of animal testing, the Basel based company attaches considerable importance to close cooperation with the TEDD group in this domain.

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## Call for Swiss Animal-free Translational Medicine

In order to drive forward the process of translational medicine in Switzerland, the *Swiss Institute for Translational and Entrepreneurial Medicine* is being set up on the Campus of the Insel University Hospital. This *Sitem-Insel, which is a not-for-profit organization,* will train specialists in translation, provide high-tech infrastructure and personnel at the interface between industry, clinical research and university hospitals, and fast-track the administrative/regulatory processes from the laboratory bench to clinical applications or bedside. A strong focus is the 3D co-culture of fibroblasts and keratinocytes at the air–liquid interface developed by CELLnTEC advanced cell systems AG. "The unique selling proposition and benefits are fully defined, animal component-free and higher quality certified products which are unique in the cell culture market around the world. They are optimally suited for clinical applications," explains Professor ***Eliane Mueller***, Head of Dermatology & Stem Cell Research at Bern University and Insel University Hospital, founder and chairperson of CELLnTEC and advisor of Sitem-Insel for stem cell research. She brings a wealth of knowledge in dermatology and skin stem cells. "Our innovations comprise full-thickness skin models with a fully natural dermal matrix that is secreted completely by the fibroblasts. This is much more *in vivo*-like and avoids the use of artificial collagen gels, which are time-consuming, variable and expensive." But the country now needs strict regulatory conditions in relation to providers at the international level for components used in the manufacture of clinical applications. "We invite all Swiss centers in this domain to support our efforts to build a future platform for cell therapy and translational medicine in order to produce clinical products of irreproachable quality with a common commitment.

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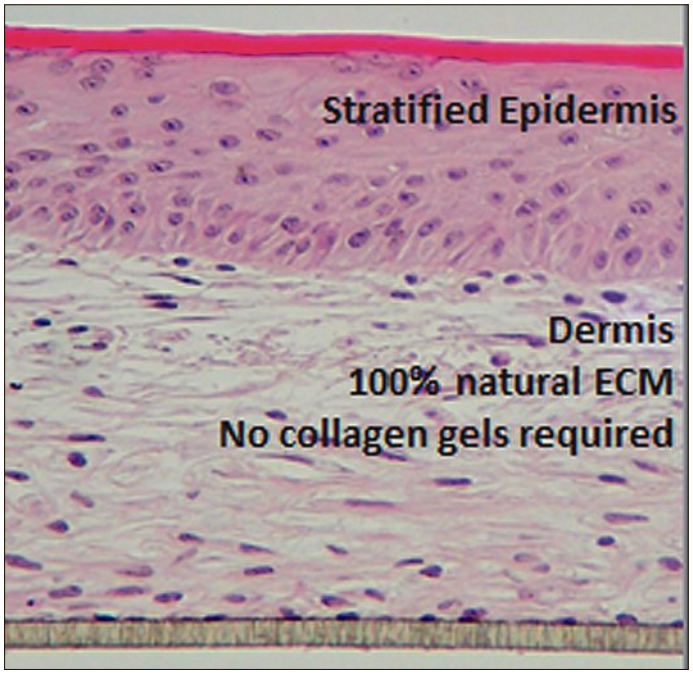


Full-thickness skin model with a fully natural dermal matrix, produced with CnT-PR-F and CnT-PR-FTAL media by CELLnTEC, advanced cell systems AG, Bern. Picture CELLnTEC.

## Let's Close Ranks!

In bringing together representatives from the cell culture industry and applied scientists, Professor ***Michael Raghunath*** wants to take a closer look at the possibility of serum-free cell culture. He has the welfare of animals very much at heart, as he was for many years a member of the NUS Institutional Animal Care and Use Committee IACUC, evaluating the work with animals, monitoring animal welfare at the university and inspecting animal holding facilities. "If an animal is suffering stress or pain, this could affect the results of a test," he states. "It therefore makes sense that – if a test with animals cannot be avoided – they must be treated with care and kept in the best possible conditions. In the case of FBS (Fetal Bovine Serum), for many decades an invaluable and essential admixture to animal and human cell culture media, we are entitled to ask whether this serum is still strictly necessary today, as we know about all its negative effects." When in doubt, leave it out? Unfortunately, it's not as easy as it looks. "Without serum, cells do not thrive, so replacement factors must be found that keep the cells going," recalls Michael Raghunath. But this will not be easy, as this is cell type-dependent, and we have over 200 different types of cells in our body! "Each component has to originate from animal-free substances, which requires full documentation," explains the Head of Cell Biology and Tissue Engineering. But this workshop has shown us that these problems can only be solved if all partners join a network that is focused on cooperating and sharing skills, knowledge and experience. "We want to contribute to animal-free research by developing, implementing and teaching the methods of stem-cell culture and the realization of relevant, living tissue models for testing, diagnostics and therapy. Do you want the same as us? Simply contact us!"

Prof. Eliane Mueller and the 'driving forces' behind the TEDD network: Dr Ursula Graf-Hausner, Board member, and Prof. Michael Raghunath, Director (left to right). Picture ZHAW.

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