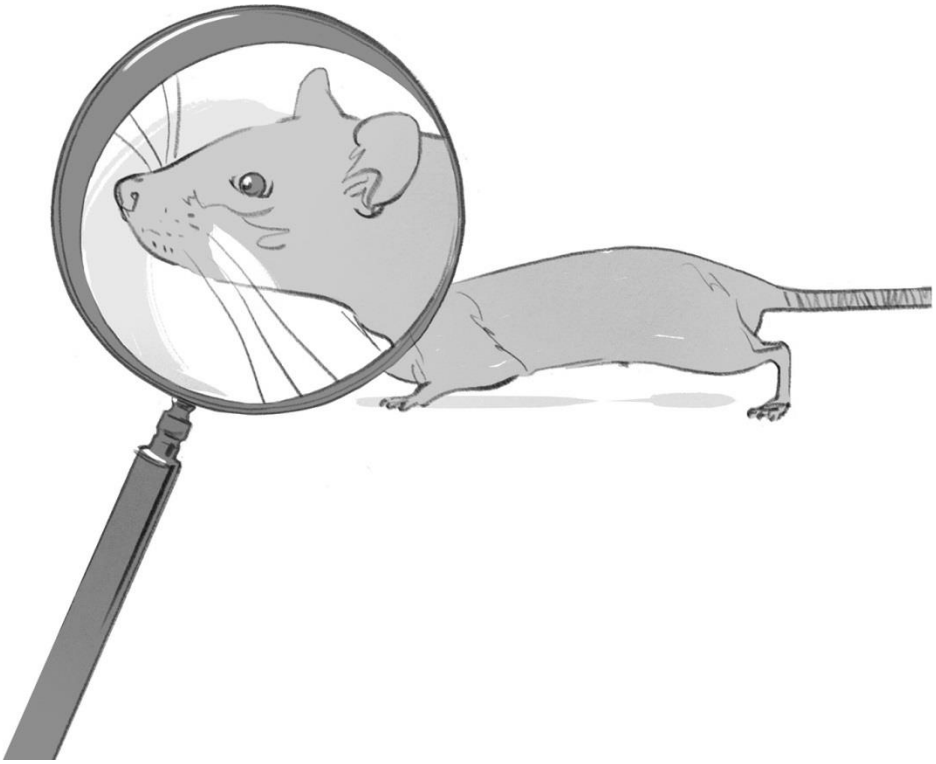
Presentations of the 11th Conference
on Animal Testing

# "The 3R competence centre (3RCC) – better research with fewer animal experiments?"



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**Speakers at the 11th SAP Conference on Animal Testing**
**"The 3R competence centre (3RCC) –**
**better research with fewer animal experiments?",**
**held at Hotel Arte, Olten, Switzerland, on 18th May 2018**


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# Introduction

Dr Julika Fitzi-Rathgen MLAW, Specialist unit animal experiments, Swiss Animal Protection SAP, at the 11th SAP Conference on Animal Testing, “The 3R competence centre (3RCC) – better research with fewer animal experiments?”, held on 18 May 2018 in Olten, Switzerland

The launch of the new 3R competence centre (3RCC) means that after more than 20 years, the 3R principles are finally to be enforced as set out by legislators under Art.22 of the Animal Protection Act back in 1993: The Confederation shall work with universities and industry to promote and support the development, recognition and application of methods that replace animal experiments, that require fewer test animals, or that cause less distress to the animals involved.

Replacement methods have hardly been used in Switzerland up to now despite the fact that they have proven economic and scientific potential and are cheaper and faster. That is set to change. On a scientific and economic level, replacement methods offer much greater possibilities and uses than animal experimentation. It is no coincidence that the EU and U.S. are investing considerable sums in developing and implementing such methods.

For animal lovers and animal welfare campaigners the key question is whether the new competence centre will lead to a significant decline in animal experiments. And secondly whether it will successfully link up industry and higher education institutions in such a way that the 3Rs, in particular the replacement methods, become a priority in research activities. Academic research in particular has seen increasing numbers of animal experiments in recent years and should now take a more active role in this area. We will consider these and other aspects with knowledgeable speakers from Switzerland and abroad at our conference.

# What the FSVO expects of the 3RCC 3R Competence Centre

Dr Kaspar Jörger, Head of Animal Welfare, Federal Food Safety and Veterinary Office, at the 11th SAP Conference on Animal Testing, "The 3R competence centre (3RCC) – better research with fewer animal experiments?", held on 18 May 2018 in Olten, Switzerland

The 3R principles[1] (replace, reduce, refine) should be implemented in every animal experiment. In Switzerland, researchers are obliged to minimise the number of animals used for animal testing. Alternative methods to replace animal testing should be used where available. Where animal experiments are absolutely necessary, the suffering of animal subjects should be kept to an absolute minimum.

All stakeholders, researchers, research funding agencies, the pharma industry and public authorities are working together to replace animal experiments, to reduce the number of animal experiments performed and to develop animal experiments that cause less pain and suffering. To support the implementation of the 3R principles, the Federal Council recommended the creation of a national 3RCC[2] competence centre.

Following a number of workshops, the Conference of Rectors swissuniversities was mandated by the federal government (State Secretariat for Education, Research and Innovation SERI and the FSVO) to develop a structural concept for a new national 3RCC. The new 3RCC was set up in March 2018. It is designed as a network of 11 higher education institutions and is supported by the institutions themselves, the pharma industry (Interpharma), the federal government and Swiss Animal Protection SAP.

**The FSVO has high expectations of the new 3RCC, in particular with regard to the key elements of education, communication and 3R research.**

The core element in ensuring effective and sustainable improvements to the welfare of laboratory animals and in reducing the number of test animals is sound **education and training** of researchers. The 3RCC's close links to universities will allow the topic of 3Rs to be included in the curriculum for students on all science and medicine courses from an early stage. The aim is to establish a 3R culture in Swiss laboratory animal facilities, research institutes and laboratories.

To achieve this, the 3RCC needs to develop and implement a 3R training strategy, which should take into account the different education and training formats and ensure coordination between existing university 3Rs teaching programmes.

This key role in education, training and development will allow the 3RCC to become a centre of expertise on the animal-friendly handling of laboratory animals and more broadly to establish itself in the field of 3Rs as a knowledge and experience sharing platform for the animal testing community. The 3RCC needs to develop a communication concept which should include the establishment of a professional contact point for the various stakeholders and which in future will allow structured **communication** with stakeholders (students, researchers, the public, the media, public authorities and policymakers).

[1] 3R principles – Replace, Reduce, Refine

[2] Future of the 3R research foundation and alternative animal testing methods, Federal Council report in response to the postulate 12.3660 of the National Science, Education and Culture Committee of 17.08.2012

This active internal and external communication will ensure maximum transparency both within the research community and among the general public. Finally, we expect the 3RCC to develop international links with other 3R competence centres in Europe and worldwide to share knowledge, experience and methods.

A **3R research** strategy should be developed to identify and initiate high-quality competitive research projects that take into account all areas of the 3Rs (replace, reduce, refine). Particular importance should be attached to projects that develop new approaches or technologies right through to implementation and which are not supported by other funding instruments (such as those of the Swiss National Science Foundation). The focus should clearly be on researching alternative methods. In the regulatory field, the 3RCC should act as a catalyst for the implementation of non-animal methods. As long as animal experiments are unavoidable, the 3RCC should support studies and projects that develop animal-friendly methods that aim to significantly and sustainably reduce the suffering of laboratory animals. The 3RCC should also promote methods that aim to optimise the number of animals used in order to obtain meaningful research findings.

The 3RCC should develop suitable evaluation instruments and key indicators for the field of 3Rs to measure and monitor the progress made in teaching and research. In addition, a set of basic principles should be established defining how “unpublishable” results in all 3R research fields should be managed.

The FSVO looks forward to working closely with the 3RCC to see progress in the implementation of the 3R principles and to support the activities of all stakeholders.

# Swiss 3R Competence Centre: Advancing research & education on 3Rs

Dr Chantra Eskes, Director of 3RCC, Bern, at the 11th SAP Conference on Animal Testing, “The 3R competence centre (3RCC) – better research with fewer animal experiments?”, held on 18 May 2018 in Olten, Switzerland

The Swiss legislation on the protection of animals requires that all person taking care of animals, takes as much as possible into account their needs and ensure their wellbeing as far as the scope of their use allows it. Considerable progress took place in the last decade at an international level for the refinement, reduction and replacement (principles of 3Rs) of animal experimentation for regulatory purposes. On the 27 March 2018, the Swiss 3R Competence Centre (3RCC) has been founded to further promote the principles of 3R in the areas of research and education.

Under the presidency of Dr Kathy Riklin, member of the Swiss National Council, the 3RCC represents an association of academia, industry, regulators, government and animal welfare association including the eleven most important Universities and Higher Education Institutions from Switzerland, the Swiss association of pharmaceutical industry (Interpharma), the Swiss Federal Food Safety and Veterinary Office (FSVO) and, the Swiss Animal Protection. The 3RCC also benefits from an important support from the Swiss State Secretariat for Education, Research and Innovation (SERI), as it represents a scientific centre of national importance working on a non-commercial basis according to article 15 of the Federal Act on the Promotion of Research and Innovation (RIPA).

Having its offices kindly hosted by the University of Bern, the Swiss 3R Competence Centre will subsidize scientific projects of quality and establish an educational program and communication strategy to promote the principles of 3Rs. A first call for scientific projects is planned for late 2018. In addition, through its educational program and communication strategy, the centre aims at making accessible to all those involved and/or interested on animal experimentation, up-to-date information on alternative methods to animal experimentation. Finally, the 3R Competence Centre will monitor progress made regarding the implementation of the principles of 3Rs in Switzerland and will offer its services to authorities, teaching bodies and other interested parties willing to gain additional information on the principles of 3Rs and on alternative methods to animal experimentation.

# The Berlin-Brandenburg Research Platform BB3R – research and graduate training since 2014

Prof Monika Schäfer-Korting, Institute of Pharmacy, Freie Universität Berlin, at the 11th SAP Conference on Animal Testing, "The 3R competence centre (3RCC) – better research with fewer animal experiments?" held on 18 May 2018 in Olten, Switzerland

The Berlin-Brandenburg research platform BB3R, which was set up at the FU Berlin in 2014, pools 3R expertise in the Berlin-Brandenburg region and promotes systematic research in this field. The integrated graduate school is the world's first to offer a structured qualification in the 3Rs for young scientists, PhD students and junior professors. The platform received support from Germany's Federal Ministry of Education and Research in the form of seed capital.

Eleven founding members conduct research in the fields of skin disease models, immunology, human-on-a-chip, nanotoxicology, in silico analysis of active compounds and drug design (reduction/replacement). For animal experiments that are not replaceable, the platform develops refinement measures and studies the impact of multiple experiments, which also aim to reduce the numbers of laboratory animals. Besides this group of experienced scientists, the platform has also comprised PhD students and three junior professors from the outset. The consortium is boosted by nine renowned scientists who are associate members. The first scientists to complete the graduate programme already hold senior positions or have been offered professorships and can therefore pursue their 3R- research in Germany and internationally.

In addition to the research project, the graduate programme also includes regular PhD symposia, 3R seminar series and annual spring schools. Particular emphasis is placed on teaching students about all aspects of the 3Rs. The programme is organised under the aegis of the FU Berlin's Dahlem Research School (DRS), which offers courses on general skills (e.g. presentation, statistical analysis, good scientitfic practice). External PhD students are also accepted at the graduate school provided they work in one of the 3R fields and meet the DRS quality requirements.

Below are some examples of the consortium's research activities.

The Schönfelder project team is working on the development of individualised pain management for mouse strains as mice are currently the most widely used laboratory animals. Based on the results of this study, it should be possible to give more precise dosage recommendations for Buprenorphine for different mouse strains in order to keep the pain experienced by mice during experiments to an absolute minimum (refinement).

The Schäfer-Korting project team is building tumour models of non-melanoma skin cancer and head and neck tumours to study the absorption and effect of cytostatic drugs. In these cases, the translatability of results from animal experiments is by far the lowest. This is to be counteracted with an integrated test strategy, where in the preclinical phase a drug candidate is initially tested for tolerability and suitability on a 3D model. Only the substances that are successful in these tests would then have to be tested for tolerability on animals in order to exclude adverse effects as far as possible when first used on humans.

# Human lung cultures as an example of research at the new Charité 3R Centre

Prof Stefan Hippenstiel, Medical Clinic, Charité - Universitätsmedizin Berlin, at the 11th SAP Conference on Animal Testing, “The 3R competence centre (3RCC) – better research with fewer animal experiments?”, held on 18 May 2018 in Olten, Switzerland

Inflammation of the lungs (pneumonia) is one of the five most common causes of human death worldwide and is a widespread disease in Europe. Studies involving large patient cohorts show that the mortality rate of this disease has remained unchanged at around 13% for over sixty years. This is especially striking considering the significant progress made in basic research, the advancements in intensive care medicine and the availability of a number of pneumonia vaccines.

The bacterium *Streptococcus pneumoniae* remains by far the most common isolated pathogen. Zoonotic viral pneumonia pathogens in particular, such as influenza viruses and SARS and MERS coronaviruses, still show significant epidemic and pandemic potential.

If we want to study these pathogens, we should bear in mind that *Streptococcus pneumoniae* is a strictly human pathogen, while the viruses mentioned above exhibit strong species specificity. Studying many of these pathogens using animal models therefore has limited relevance to humans; for some the available models are very insufficient (*1, 2*). These examples show that animal models have significant limitations in their translatability to humans, at least in relation to certain diseases. In addition, there are major differences in key physiological and pathophysiological characteristics between species. The use of experimental subjects that are as homogenous as possible and various other aspects partly explain why many methods that are successful in basic and preclinical research fail when they are translated into clinical practice. Besides ethical questions, there are thus many scientific reasons to actively develop viable alternatives to animal testing. The new Charité 3R Centre therefore aims to consolidate the faculty’s activities in all areas of the 3Rs with a focus on the systematic development of alternative methods. This involves seeking interdisciplinary cooperation with local and external partners from the outset. The stakeholders believe that development of the models themselves poses a major scientific challenge and that significant efforts need to be made to standardise and distribute them (e.g. cryopreservation).

As an example, a cultured *ex vivo* infected human lung tissue is being presented here. This allows us to study the relevant infection processes of bacteria (*3-6*) and viruses (*7-11*) in pneumonia. It should be noted that in its current form this model obviously also has inherent limitations as it does not take account of breathing or circulation. But it does allow us to compare the replication of viral and bacterial pathogens using clinical isolates without the need for adaptations, which enables us to identify the true pathogen-specific virulence factors. Using spectral confocal microscopy imaging, we can identify the pathogen tropism and local alveolar damage. This high spatiotemporal resolution microscopy allows the movement and localisation of mitochondria and the development of apoptosis in three-dimensional tissue to be observed live in the presence of fluorescent pathogens. Traditional methods, for example to measure inflammatory response (e.g. interferons) are also used. We can analyse molecular mechanisms in differentiated tissue using e.g. GFP-tagged proteins which are transmitted via viral transduction. On the whole, this method not only constitutes an alternative to traditional animal testing, it also (subject to its own limitations) allows us to obtain information of great biomedical relevance, which animal models cannot provide.

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# The Tox21 concept: toxicology without animal experiments

Prof Horst Spielmann, Institute of Pharmacy, Freie Universität Berlin, at the 11th SAP Conference on Animal Testing, "The 3R competence centre (3RCC) – better research with fewer animal experiments?", held on 18 May 2018 in Olten, Switzerland

Because the development of new drugs using animal experiments is becoming increasingly expensive and because many such drugs have proven toxic or ineffective when used on humans, experts from the US Academy of Sciences tried to develop a new scientific concept for drug development free from the ethical problems of animal testing. The results were published in 2007 in 'Toxicity Testing in the 21st Century – A Vision and a Strategy'. In it, the experts concluded that in a not-so-distant future all routine toxicity testing would be conducted in human cells or cell lines in vitro by evaluating perturbations of cellular responses in a suite of toxicity pathway assays. In other words, animal experiments would be replaced by studies using human cells and tissue.

A key element of this Tox21 concept was the suggestion that the toxicity of foreign substances, drugs and endogenous substances, such as hormones, is based on adverse outcome pathways (AOPs).

As it has been possible to grow human cells and tissue under physiological conditions for 20 years, the AOP concept was extensively tested and confirmed by scientists at universities, in industry and in public agencies The EU AXLR8 project, which I coordinated at the FU Berlin from 2009 – 2012, also contributed to these efforts in Europe. An initial success based on the AOP concept was the development of new OECD testing methods for skin sensitisation which used human cells and tissue so that animal experiments were no longer required. The Tox21 concept led to a paradigm shift internationally, and AOPs now have to be taken into account when developing new toxicological test methods in all areas of pharmacology and toxicology, namely using human cells and tissue. Of course, this is also resulting in the replacement of animal experiments that were previously compulsory.

Multi-organ chips, on which several miniature human organs can be grown, have played a major part in this advancement. They are now used in the development of new drugs and to assess the risks of ingredients of cosmetics for which animal experiments are no longer permitted in Europe.

The US Academy of Sciences has since reviewed the Tox21 concept and in 2017 published the study "Using 21st Century Science To Improve Risk Related Science". In it, the experts conclude that the Tox21 concept has clearly improved the quality of risk assessment for the protection of human health and the environment. To put this insight into practice, the major US federal agencies – FDA, EPA and NIH – launched extensive support programmes for the development of new safer drugs and chemicals in consumer products at the end of 2017. It is hoped that Europe and other industrial nations will soon follow suit, as the Tox21 concept confirms the scientific superiority of animal-free methods.

# In search of alternatives to foetal bovine serum – light at the end of the tunnel

Prof Gerhard Gstraunthaler, Division of Physiology, Medical University of Innsbruck, at the 11th SAP Conference on Animal Testing, "The 3R competence centre (3RCC) – better research with fewer animal experiments?", held on 18 May 2018 in Olten, Switzerland

The use of serums as supplements to culture media has long been routine practice in cell and tissue culture. Serums, in particular foetal bovine serum (FBS), supply cultures with hormones, growth and attachment factors, binding and transport proteins, additional amino acids, vitamins and trace elements.

However, the use of foetal bovine serum also has a number of disadvantages. Serums may contain bacterial toxins (endotoxins) and undesirable microorganisms such as bacteria (including Mycoplasma), viruses and prions. Furthermore, there are enormous seasonal and geographical variations in the qualitative and quantitative composition of individual serum batches, which often necessitates costly and time-consuming batch tests. The serum thus introduces an undefined mixture of biologically active substances into a defined culture medium.

Foetal bovine serum is a by-product of the beef industry. As such, the serum market is dependent on many external factors. The question is therefore increasingly being asked as to whether the worldwide demand for foetal bovine serum in research and the biotech industry can be met at all. Scandals concerning adulterated foetal bovine serum that have recently come to light have further heightened concerns about the purity and quality of serums.

The most serious drawback, however, is the serum extraction method. Foetal bovine serum is harvested from the foetuses of pregnant cows. It is estimated that approx. 800,000 litres of foetal bovine serum are required every year, which equates to 2 million bovine foetuses. The ethical concerns around serum extraction have gained traction in recent years and a range of alternatives have been identified in order to reduce the use of, and/or fully replace, foetal bovine serum to lower the annual consumption figures for bovine foetuses in line with the **3Rs.**

Despite many innovative approaches and the development of serum-free media for a wide range of cells, the use of foetal bovine serum still remains the method of choice in cell culture.

To avoid the disadvantages of using serum, to create defined and controlled culture conditions and for animal welfare reasons, the search for alternatives to the use of serum in cell culture has intensified in recent years. We now have a promising solution on the horizon.

**Platelet lysates as an alternative to foetal bovine serum (FBS)**
Human platelet lysates (hPL), which are enriched with platelet-derived growth factors, are the latest development. Serum contains a wide range of mitogenic growth factors, which are released from activated platelets during the coagulation process. Many of these factors were identified early on as essential mitogens in serum-free culture media. Following on from this observation, human platelet lysates have established themselves as an adequate substitute for foetal bovine serum in a wide range of different culture systems in recent years. Human platelet lysates are derived from expired donor platelets collected in blood banks. Donor platelets have a shelf life of just five days within which they may be used clinically. This means that donor platelet concentrates are regularly available.

The use of human platelet lysates (hPL) in cell culture should be considered in the context of platelet physiology. Platelets produce a range of growth factors, which they store in

their α-granules and release when they are activated. These factors play a key role in stopping bleeding and in the subsequent wound healing process.

It is well known that the coagulation process in the harvesting of raw serum is critically important to the quality of the serum. It can therefore be assumed that the factors detected in the serum that are essential to the proliferation of cultured cells, such as EGF, PDGF, FGF, TGF-β and VEGF are of platelet origin. The high level of specific growth factors therefore makes human platelet lysates an excellent, if not fully defined, substitute product for cell and tissue culture.

As mentioned above, hPL are derived from expired platelet concentrates obtained by apheresis. Plateletphereses are carried out in certified blood banks. This means that once the shelf life has expired, a quality-tested starting product manufactured in accordance with European guidelines and certified for therapeutic use (platelet donation) is available. The donor platelets are washed, resuspended in a saline solution and lysed in a simple freeze-thaw process. The lysates can be added as a serum replacement to basal media, such as MEM, DMEM, DMEM/Ham's F-12 and RPMI-1640 in a concentration of 5% (v/v).

In addition, human platelet lysates are a culture system based purely on human factors. Such systems are free from any animal-derived components and are thus particularly suited to stem cell culture and tissue engineering.

Foetal bovine serum cannot be extracted directly or manufactured. However, the potential availability of expired donor platelet concentrates from blood banks and the ease with which lysates can be produced give us reason to be optimistic that this innovative and successful serum replacement method will soon be used more widely in cell culture laboratories.

# Recombinant antibodies (research, production and implementation)

Prof Pierre Cosson, Department of Cell Physiology and Metabolism, University of Geneva, at the 11th SAP Conference on Animal Testing, “The 3R competence centre (3RCC) – better research with fewer animal experiments?”, held on 18 May 2018 in Olten, Switzerland

Replacing animal experiments in biomedical research is a long and complex process. For example, recombinant antibody technology has allowed antibodies to be produced without the use of animals for more than 20 years. Application of this technology could significantly reduce the number of animals used in laboratories, while facilitating research tasks. However, it has not yet extended to basic research laboratories in the biomedical field, chiefly because of its relative sophistication and cost. Our overall objective is to promote the replacement of animal-derived antibodies with recombinant antibodies produced entirely in vitro.

In 2014, we opened a university centre in Geneva to offer access to recombinant antibody technology to basic research laboratories, and to reduce the use of animals in research. The centre focuses on the discovery of new recombinant antibodies, of which it has produced hundreds (see Figure 1).

A new project that is currently underway involves creating a complete database of all recombinant antibodies that have been discovered to date. This database will be linked to a production centre, which will provide access to all these antibodies. Our long-term vision is to create a completely open centre which will produce antibodies in vitro for the global scientific community. This example illustrates the potential and the difficulty of implementing a new technology to replace animal experiments.

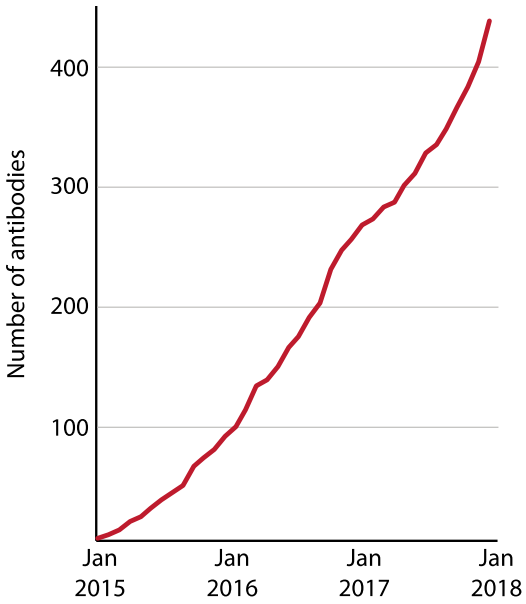


Figure 1: Number of recombinant antibodies available at the Geneva Antibody Facility. https://www.unige.ch/antibodies

# The Scar in the Jar – an in vitro system to test antifibrotic substances

Prof Michael Raghunath, Head of Centre for Cell Biology and Tissue Engineering, Zurich University of Applied Sciences ZHAW, at the 11th SAP Conference on Animal Testing, "The 3R competence centre (3RCC) – better research with fewer animal experiments?", held on 18 May 2018 in Olten, Switzerland

Scars are recognisable because they look different from normal tissue. Scar tissue is the result of a rapid repair process and describes the accumulation of collagen fibres at the site of a wound healing reaction. This reaction did not evolve to look nice, nor to restore full function, but to rapidly restore tissue cohesion (Mother Nature's quick fix). Scar formation follows a relatively predictable process: following an injury, i.e. a break in the local tissue architecture (scratch, cut or crater), there is bleeding followed by haemostasis. Then comes the inflammatory phase, during which phagocytic cells seek out the lesion, clear out the bacteria and digest the damaged tissue; repair cells, fibroblasts and endothelial cells are then drawn to the wound (proliferative phase). At this point, the wound is a veritable construction site, with demolition and reconstruction work going on, and where in the end construction prevails. The building material is extra-cellular matrix, mainly collagen. Collagen forms fibre systems, which fill and bridge the wound defect, allowing cells to migrate and form a closed roof. The collagen fibres are more densely packed than normal tissue, which is why we can recognise scars with the naked eye as well as under a microscope. During the various phases of wound healing, the regeneration phase leads to wound closure. The collagenous scar then matures; it hardens but decreases in size. Surgical scars, for example, require at least a year before they turn from red to white. Every surgical intervention involves scarring in all affected layers of tissue.

Scars on the skin can significantly reduce the appearance and function of the skin's mobility and elasticity. However, if scarring encompasses a whole organ, it can be life threatening. The first stage in scarring is inflammation. This means that even without an actual injury, chronic inflammatory processes (toxic substances, virus infections) can trigger scarring, which affects entire organs, such as the liver and lungs. The number of new cases (incidence rate) of cirrhosis of the liver (a special designation for liver fibrosis) is 250 per 100,000 people per year in industrial nations. Besides alcohol-related cirrhosis of the liver, the chronic hepatitis virus is the second most common cause in industrial nations, causing 20–25% of cases. In Africa, the hepatitis virus – predominantly Hepatitis C – is the most common, causing 90% of cases.

In order to stop fibrosis and local scarring, we need to be able to curb inflammation and intervene in collagen metabolism. Ideally we would need to be able to stop either collagen excretion or deposition around the producing cells. Wound healing studies conducted on conventional small laboratory animals such as rats and mice have to bear in mind that skin wounds in these animals heal through contraction rather than through scarring. Certain forms of scarring, such as keloids, appear to be specific to humans and cannot be perfectly reproduced in animal experiments. Liver fibrosis models are usually produced in rats by administering substances that are toxic to the liver, such as carbon tetrachloride. Such animal experiments are time consuming and complex.

Cell culture systems therefore lend themselves to pre-testing anti-fibrotics in vitro. Unfortunately, collagen deposition, i.e. the formation of a fibre skeleton around cells, is very inefficient in the standard culture medium. This is because the enzyme BMP-1, which trims the procollagen released into the culture medium to make collagen, works very slowly in an aqueous environment. Only collagen, not procollagen, can clump together to form insoluble fibres. BMP-1 is therefore a limiting factor in collagen matrix building in vitro. Most academic and pharmaceutical teams are still not aware of this. The standard culture medium is

an artificial, highly aqueous environment in which cells are not usually found. In reality, the interiors of cells are filled with a large number of macromolecules, and the microenvironment of cells is dominated by macromolecules. There is little free water, either inside the cells or in their environment. This condition is called macromolecular crowding. We have been developing artificial macromolecular crowding for cell culture for 15 years (Chen et al 2011). Adding macromolecules (usually sugar polymers) accelerates the activity of the procollagen-trimming enzyme BMP-1 and therefore results in rapid conversion of procollagen to collagen and thus to efficient collagen deposition in vitro for the first time. Other enzymes which chemically connect and therefore stabilise the collagen skeleton are also accelerated, as is the polymerisation of collagen molecules (fibril formation). Using macromolecular crowding we have created a smart culture system in which the entire scar cascade can be reproduced in a petri dish, and in which the relevant biochemical and enzymatic processes function efficiently.

We have successfully tested the Scar in a Jar system and in the case of anti-fibrotic substances, we were able to prove beyond doubt to two different pharma companies which substances work and which do not (Chen et al. 2009). If one of the companies had applied macromolecular crowding in vitro, the ineffectiveness of the substance would have become clearer and would have made subsequent animal testing unnecessary. In retrospect, it became clear why the results of the animal experiments produced unclear results at considerable cost. The Scar in a Jar thus fills a test gap in the development of anti-fibrotics and has been adopted by industry: since 2011 it has been successfully used by GlaxoSmithKline for in vitro testing of substances to treat pulmonary fibrosis.

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# Microphysiological systems in translational research – applications and perspectives

PD Dr Alexander S. Mosig, Institute of Biochemistry, Jena University Hospital, at the 11th SAP Conference on Animal Testing, “The 3R competence centre (3RCC) – better research with fewer animal experiments?”, held on 18 May 2018 in Olten, Switzerland

The role of the microbiome in the function of the human gut has attracted a great deal of attention in recent years. It is becoming increasingly clear that a physiological gut-microbiome interaction is essential to good health. If the composition of the microbiome (defined as the full array of microorganisms that live on and in humans) becomes physiologically unfavourable or even dominated by pathogenic bacteria (dysbiosis), this has a significant impact on the development and progression of diseases such as inflammatory bowel disease, organ failure in critically ill patients and sepsis.

A disruption of epithelial and endothelial intestinal barrier function is a typical pathological change in acute sepsis. Different mechanisms are currently under discussion to explain this. It is assumed that both signalling processes of an excessive immune response and direct interaction of the microbial pathogens with the epithelium and endothelium cells lead to a disruption of the barrier function. As a result of the subsequent systemic inflammatory responses and infections, multiple organ dysfunctions occur, with the liver being one of the first organs affected due to its direct connection to the gut. The liver is home to around 80% of the body’s macrophages. Circulating monocytes constantly patrol inside the hepatic vascular system for pathogen-associated molecular patterns (PAMPs). Once PAMPs are detected, they are migrated into the liver tissue. To avoid adverse immune responses, endotoxins from the microbiome are tolerated by the liver within defined limits under physiological conditions. However, efficient defence against infection requires strict regulation of the inflammatory response on the one hand, and immunotolerance on the other. In this respect, macrophage activation is a central aspect as these cells are able to mediate both inflammatory responses to control bacterial infections, and tolerance of physiological endotoxin concentrations.

Due to the limitations of currently available *in vitro* methods, we often revert to animal models to study the physiology of gut-liver interaction and how this is deregulated in cases of infection and sepsis. Important anatomical and genetic similarities between mice and humans, as well as low husbandry costs, high reproduction rates and a short lifecycle compared with other mammals, have led to the mouse model being widely used in inflammation research in recent years. However, on account of obvious differences in diet, habitat and body size between mice and humans, there are still significant differences between the species. For example, species-specific metabolic requirements have resulted in significant evolutionary differences in the structure and microanatomy of the gut, and the function and composition of the immune system.

These significant limitations have led us to develop microphysiological models of the human gut and liver, with which we can describe the complex interactions in detail between the two organs on a molecular and cellular level in physiologically relevant conditions *in vitro*. The organ models are already routinely used as a central tool to study the pathophysiology of organ failure in sepsis, and to develop new therapeutic approaches for patients. At the same time, they are paving the way for the permanent replacement of animal testing in research at the Centre for Sepsis Control and Care at Jena University Hospital and in interregional research projects with academic and industrial research partners. I look forward to presenting this organ model and the associated organ-on-a-chip in more detail during my talk.

## Notes