The workshop on replacing animal use in teaching physiology and pharmacology at Eastern European universities was held on the 7th and 8th of October 2009 in Belgrade, Serbia.

The two-day event was organised by David Dewhurst from the College of Medicine & Veterinary Medicine, University of Edinburgh, Scotland and Zvezdana Kojic from the Institute of Physiology, Medical School, University of Belgrade, Serbia. The workshop brought together more than 20 teachers of pharmacology and physiology from Bosnia and Herzegovina, Bulgaria, Czech Republic, Estonia, Greece, Lithuania, Macedonia, Poland, Rumania, Serbia, Turkey and the Ukraine.

The aim of the workshop was to introduce the participants to current and new techniques of teaching physiology and pharmacology in higher education. It was also strongly focused on how the successful introduction of alternatives into the curricula can significantly reduce the number of animals used for teaching purposes in physiology and pharmacology at Eastern and Central European universities.

The workshop opened with a public event in the lecture theatre of the physiology institute. Zvezdana Kojic gave a welcome speech and briefly presented the project to the audience, emphasizing the importance of the workshop for the dissemination of the 3R concept in teaching. Vladimir Bumbasirevic, dean of the school of medicine and head of the institute, introduced the institute and its activities and expressed his gratitude to the participants for coming to Belgrade to share their knowledge and experience and to improve their teaching techniques and output. David Dewhurst gave a presentation on new techniques in teaching and learning based on the established and well-accepted model of contemporary e-learning at the University of Edinburgh.

The workshop continued the next day in the conference room of the Hotel Prag. The session was opened with a presentation by David Dewhurst entitled “Innovation and teaching in Physiology and Pharmacology”. It provided an overview of alternatives to animals used in teaching in the UK and presented opportunities and challenges in teaching using computer-based alternatives to animal testing. Zvezdana Kojic continued with a synopsis of a successful small-scale pilot workshop held in 2006-2007 to introduce computer-based alternatives in a number of universities in South Eastern Europe. Steve Quirrie, director of the consultative bureau for international projects in Serbia, gave a brief presentation about project funding opportunities, primarily in the EU. He introduced a model of how project proposals should be planned and submitted in order to compete for successful funding at the European level. Goran Krummenacher, a representative of the Doerenkamp-Zbinden Foundation for Alternatives in Biomedicine, presented the foundation’s activities and aims as well as its funding and evaluation procedures.

The discussion elements of the workshop addressed many opportunities and challenges in introducing alternatives to animal use to physiology and pharmacology teaching. They also encouraged the sharing of ideas, experiences and different approaches among the participants.

A network and a web platform were established with the aims of maintaining the co-operation among the participants and allowing the easy exchange of ideas, future plans and projects. Further, all the participants agreed to evaluate the number of animals and the kinds of procedures employed for teaching in their departments as soon as possible in order to propose adequate alternatives. Some ideas on the name of the network, possible funding options and project proposals were discussed and agreed upon.

This workshop established a solid platform for further cooperation among workshop participants and promotion and implementation of alternatives to animal use in teaching physiology and pharmacology in Eastern and Central European universities. More such events and efforts are necessary to accelerate the successful and sustainable reduction and replacement of animal use in the teaching of biomedical sciences.

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1 This event was fully sponsored by the Doerenkamp-Zbinden Foundation, an internationally active foundation based in Switzerland, which is dedicated to the development and promotion of alternatives to animal use in biomedical research and education. The activities of the foundation are entirely in accordance with the 3R principle (replacement, reduction and refinement) in the field of biomedicine. Up to now it has supported and initiated a number of projects in the field of alternatives to animal use. It has also established 6 endowed university chairs for the development and promotion of alternatives to animal testing.
DECHHEMA conference “Organotypic Tissue Culture for Substance Evaluation”

Potsdam, Germany, 22-25 September, 2009

High-tech tissue culture models – loaded with multiple cell types on nano-optimized substrates, vascularized and even with access to reservoirs of stem cells – are about to become a new reality in preclinical testing, both regulatory and especially non-regulatory. Even though these complex models today account for a very small percentage of all preclinical testing models, a combination of factors will drive more of these models to market in the short time frame of three to five years. Factors that justify this aggressive time frame include:

- Regulatory pressure from legislation such as the European REACH;
- Demonstrated success of multiple-cell-type models such as 3d skin models; and
- Burgeoning academic pursuit of ever more realistic tissue culture models.

Add the cost savings likely to arrive via outsourcing as soon as these models are reduced to practice and we have the perfect recipe for the near-instant growth of an industry: just add defined culture medium.

REACHing for new regulations

External drivers for the acceleration in animal alternatives include both policies and end markets. On the policy side, EU legislation banning animal tests for acute toxicity has already taken effect, with a commensurate boost in the market for non-animal skin tests especially for cosmetics. But with two laws, the European Union has put into play a much larger market. The so-called REACH legislation, agreed in 2006, demanded retrospective testing of all chemicals on the market. (REACH stands for Registration, Evaluation, Authorisation and Restriction of Chemical Substances.) And the 2003 amendment to the 1976 EU cosmetics directive, which eliminates all testing of cosmetic ingredients on animals by 2013, puts enormous pressure on cosmetics and consumer products manufacturers to replace animal tests.

The impact of these laws will be far-reaching. The cosmetics directive, for example, will apply to products developed in Europe and to imported products marketed in Europe. And REACH has already ‘reached’ beyond Europe’s borders. “The European Union will hold test systems to a higher standard and this in turn will impact FDA,” said Lynn Allen-Hoffmann, CEO and CSO of the US-based skin testing company Stratatech Corporation. “I think the US requirements will become more stringent both regarding sourcing of material (for in vitro tests) and for endpoint assays.”

Though the push to replace animal tests in cosmetics has been grabbing headlines (see “Toxicity testing gets a makeover,” *Nature* 461, 158 (2009)), pharmaceutical R&D represents the real market. One 2002 publication, cited by industry insiders as authoritative, put the percentage of animal tests performed for cosmetics testing at 0.3% (Schumann, R. The Seventh Amendment to the Cosmetics Directive: what does DG Enterprise want from ECVAM? *ATLA*, 30, Supplement 2, 213-214 (2002)).

The drivers for pharmaceutical R&D to adopt alternative assays not only involve the desire to minimize use of animals; pharma predominantly wants to lower R&D costs while increasing R&D productivity. Toxicity testing places a huge burden on pharma R&D budgets without delivering a big return on investment. Tox testing costs pharmaceutical companies $1.5 billion annually, according to industry consultancy Select Biosciences (Select Biosciences Report on Toxicity Screening Markets, Feb. 2009), while 63% of drugs that fail during clinical development do so for reasons of safety.

Pharma thus has two problems to solve: improving the quality of non-regulatory testing, for example with truly predictive efficacy models, while also reducing the cost of toxicity assays and other regulatory tests at no loss in assay fidelity. These challenges will be met by the increasingly complex single-tissue and multi-tissue models under development. “In vitro companies are coming up like fungi,” said Bart de Wever, a serial entrepreneur in the skin testing space who was a senior manager at SkinEthic, sold to L’Oreal in 2006.

Skin testing shows the way

Skin testing is the successful example that will show the way to companies working on other organs. The first human skin models were developed for the purpose of tissue engineering. The scientific and product development work was successful: in the 1990s, companies like Advanced Tissue Sciences (ATS) and Organogenesis brought to market their skin products, for instance used for treatment of burns and diabetic foot ulcers, respectively. ATS even had an IPO. However, the companies did not recognize the enormous challenge they faced in obtaining true market acceptance and reimbursement for products with a high cost of goods. By 2002, all of these first-generation companies were either bankrupt or had reorganized.

More recently, other skin model companies have found products. These companies have focused less on tissue engineering and more on skin toxicity or skin corrosion. These include the French company EpiSkin (acquired by L’Oreal in 1997) and US-based MatTek (still private and independent) as well as SkinEthic (France), CellSystems (Germany), Phenion (Germany – now part of Henkel AG) and US-based StrataTech. Some of the skin models in current use are three-dimensional and are able to recreate the multilayered, multi-cell-type qualities of actual human skin. “I am very convinced that the response of this tissue is much more predictive than the monolayer response,” said de Wever. Furthermore, these 3-d skin models have become the “gold standard” for topical substance evalua-
tion, endorsed by the 30-nation OECD (Organisation for Economic Co-operation and Development).

The next big barrier to fall for skin models will be regulatory in nature. “Animal testing is still the regulatory gold standard,” said de Wever. “Many regulators do not seem to understand in vitro data. It has been (incorrectly!) explained to them that data obtained in in vitro models is ‘simpler’ and therefore less predictive,” no matter how complex the in vitro models become. This is bound to change, probably first in Europe and then elsewhere, under the growing pressure of the new regulatory regime imposed by REACH and the cosmetics legislation.

In the meantime, new science is laying the groundwork for tissue tests in many complex organs. Areas advancing quickly include nanomaterials and surface science; the addition of rare cells; and adding vasculature to multi-cell-type models.

**Nano: the next frontier**
 Biology is ultimately physics. Physics underlies every biological process: cell division, cell growth, mutation, selection and especially cells’ interaction with their environment. Therefore it is not surprising that physical factors are increasingly important as tissue culture systems increase in complexity.

In his conference-opening lecture in Potsdam, Prasad Shastri of the University of Freiburg described a paper in Nature Physics (Nature Physics 5, 606 – 612 (2009)) by the Grzybowski group at Northwestern University in which “asymmetric micro-geometries” such as micro-ratchets can be used to control otherwise random motion of cells. Imposing order on cells through passive means such as the nano-architecture of their substrates is an extremely promising area. Shastri’s own work has shown that the “nanoroughness” of a cell’s environment impacts the timing of cell cycle events such as DNA synthesis. The changes observed are the same as when growth factors are applied. “Environment is a clue we had previously ignored,” he says.

Sculpting the right nanoenvironment is even more important for drug and nutrient delivery. Monika Schaefer-Korting from the Free University of Berlin showed that uptake of opioids through the skin could be greatly enhanced beyond the standard 1-3% by loading them onto lipid nanoparticles. Michael Buchmeiser of the Leibniz Institute of Surface Modification in Leipzig showed early studies on how calcium carbonate and hydroxyapatite nanostructures could be created that delivered ideal amounts of calcium to cells growing nearby.

**Adding rare cells**
 Another frontier rapidly being mastered by tissue culture pioneers is the addition of rare cell types. “Skin consists of more cell types than keratinocytes and fibroblasts,” said de Wever. So skin modelers such as Heike Walles (née Mertsching) are adding adipocytes. These cells “store substances over a long period. If you want to study [skin] over the long term, you need adipocytes,” said Walles, who is Chair of Tissue Engineering and Regenerative Medicine at the University of Würzburg in Germany, in her conference lecture.

In a similar way, Hemant Kocher of the Tumour Biology Laboratory of the Barts & the London School of Medicine showed compellingly in his lecture that novel in vitro organotypic pancreatic tumor models could become extremely predictive if a single rare cell population – the stellate cells – were added to cultures of pancreatic cells (Am J Pathol 175(2): 636-48 (2009)). The underlying logic is striking: stellate cells, which were first isolated in 1998 by Apte et al. from rat pancreas (Gut 43: 128-133(1998)), are stromal cells and are vital for stromal reaction in pancreatic cancer. They serve as storehouses of, among other substances, vitamin A. Perhaps, Kocher reasoned, they are also beacons instructing neighboring cells to increase or decrease their proliferation or metabolic activity. Sure enough, Kocher showed that modifying stellate cell behavior can be used to alter tumor behavior. “Using this pancreatic cancer model, we can dissect out the crosstalk between tumor & stroma … and directly target the stroma,” said Kocher. This model provides a useful assay for testing drug candidates and might lead to therapies that previously would not have been identified in a cancer where virtually all traditional approaches have failed.

**Adding vasculature**
 “Single cells are destined to die.” That statement, made by Helmut Augustin of the German Cancer Research Center in Heidelberg in his lecture, neatly captures the necessity for seeding multiple cells into tissue models. But multiple cells and even multiple cell types are not enough. One must incorporate functional structures like blood vessels or even nerves if tissue models are ever to be truly life-like. This puts endothelial cells in the spotlight. The worldwide leader in vascularized tissue models is Helke Walles of the University of Würzburg. Walles’ tissue models range from skin, intestine and liver to tumor and trachea.

Vascularizing tissue grown in culture allows Walles and her colleagues to overcome the size limitation that otherwise plagues tissue engineers. “In liver and other organs with high metabolic activity,” she said, “we and others could show that cells more than 0.8mm from a blood vessel do not get enough oxygen.” Choosing pig jejunum was a choice inspired by necessity. Artificial scaffolds were limited to a vessel diameter of 3mm. “There was no way to generate a complex implant with that method,” recalled Walles. But by isolating the jejunum of a pig, the middle portion of its small intestine, and eliminating the porcine cells, Walles was able to seed endothelial cells into the remaining tubular structures and create functioning networks of blood vessels. The tracheal tissue she and her team crafted on pig jejunum from autologous human cells have even been successfully applied in the clinic, plugging holes in the trachea that resulted from trauma or surgery.

**Multicellular models: from stem cells to waste disposal**
 The most ambitious tissue models described at the conference – for either substance testing or for tissue engineering – combine all the elements described above: they are vascularized, contain multiple cell types including rare cell types and are constructed in physical environments conducive to controlled cell proliferation. One site where all these technologies are coming together is the Fraunhofer Institute for Material and Beam Technology (IWS), Dresden, Germany, coupled with the Department of Medical Biotechnology at the Technical University of Berlin.
In Dresden, a group led by Frank Sonntag is working toward a very ambitious systemic toxicity platform using a "chip-based multi-organoid culture system for research and substance testing." They are simultaneously pursuing three tissues for toxicity testing: liver, brain cortex and bone marrow.

Sonntag defined the micro-organoid as "the in vitro equivalent to the smallest functionally self-reliant unit of a human organ, such as liver lobuli, neuronal layers of the cortex, specula in the bone marrow, nephrons in the kidney or the alveoli in lung." By focusing on the needs of the functional units, for example in constructing the physical shape and scale of their microenvironment, Sonntag and his team hope to achieve the most lifelike responses yet. And by making sure to include multiple components including multiple cell types, vasculature and stem cells, he hopes to greatly extend the useful life of his "micro-bioreactors."

Sonntag’s work echoes that of Sangeeta Bhatia, a professor at the Massachusetts Institute of Technology (MIT) in Cambridge (who did not speak at the conference) whose group has made important progress toward weeks-long cultures of primary human hepatocytes, a step that might prove very useful for in vitro drug testing. However, there is one important difference: Bhatia, who has founded a company, Hepregen, is focusing for the moment on single tissue, the liver, although the principles she discovers may someday be applicable to culturing other organs.

Sonntag’s mention of micro-organoids also echoes the commercial-stage work on an “artificial lymph node” developed by Berlin-based company ProBioGen. Here too, the principle is to create the smallest possible functional unit, this time from multiple cell types via a process that Christoph Giese, the Head of ProBioGen’s department of Cell and Tissue Services, in his talk at the conference called “in vitro controlled organogenesis.”

Cells are prepared in complex co-culture (that is, with multiple cell types), perfused with the optimal defined culture medium in a 3-d macroenvironment, said Giese, then harvested and seeded onto the bioreactor platform, which includes the stromal cells also found by others to be so important. Remarkably, the system demonstrates appropriate "immune responses", both T-cell and B-cell-based, to antigens such as viral proteins presented in the medium. The system is already in use for testing substances both in normal and disease states.

As important as it is that the micro-scale, multi-tissue cultures described here be lifelike, viable and predictive, it is equally important if not more important that they also be manufactureable, scalable and ultimately available at multiple price points. It might seem early in the game to be thinking about the path to commercial success but in the end that will be the ultimate proof.

How close are these techniques to widespread application? It took 3-d skin models close to ten years, roughly from 1997 to 2007, to achieve widespread adoption. The efforts to combine multiple tissue types on a single platform – such as Sonntag’s – have begun in the past two or three years and are anticipat­ing significant milestones including full proof-of-concept in the 2011 time frame. But given the pace of development – with parallel efforts springing up in Europe and the United States, significant industry interest and experienced entrepreneurs who have created companies in the past, it is easy to believe that the pace might be even quicker.

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Doerenkamp-Zbinden Foundation establishes chair in India

Tiruchirappalli, India, 2nd October 2009

After the establishment of university chairs in Erlangen, Kon­stanz, Utrecht, Geneva and Baltimore (in this sequence) the DZF has established a sixth chair in southern India at the Bharathidasan University in Tiruchirappalli, Tamil Nadu. The official name of the chair is the “Gandhi-Gruber-Doerenkamp Chair for Alternatives to the Use of Animals in Life Science Education and In Vitro Toxicology”. The DZF is also funding the construction of the “Mahatma Gandhi-Doerenkamp Center”, which shall be completed next year.

The DZF specifically chose a university in India because there are so many students of biomedical sciences in that country: 200,000 students study biology in Tamil Nadu province alone. The numbers of animals used in education is thus relatively high. On the other hand, India is also a country in which information technology is highly appreciated and which boasts internationally acclaimed IT research. Therefore it appeared the ideal location to initiate e-learning in biomedical sciences. The use of animals in education will decrease dramatically. Developing new programmes and updating old ones will be a challenge that will carry the 3R idea into the academic culture by harnessing available skills. Such programmes will be of high interest internationally for education in biomedical sciences.

Bharathidasan University is also especially committed to com­memorating Mahatma Gandhi. The inauguration ceremony was held on the 2nd of October 2009, the 140th anniversary of Mahatma Gandhi’s birthday.

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The initiator of the founding of the new chair is certainly the biologist, Shiranee Pereira, who has worked with great dedication to establish alternative educational methods in India for many years and who organised the First Indian Congress on Alternative Methods in January 2007 (see news item in ALTEX 2/07 and Chennai Congress Proceedings in ALTEX 3/07).

This congress brought about the contact to the current chairholder, Prof. Dr. Mohammad Abdelkader Akbarsha, who celebrated his inauguration on the 2nd of October. The day before the ceremony, a meeting of the governing council and the advisory board, which will accompany the chair as determined in the contract, was held. David Dewhurst, professor for e-learning at the University of Edinburgh, UK, represents the DZF and holds seat and vote on both councils.

The inauguration commenced with greeting addresses given by the vice chancellor of the university, M. Ponnavaikko; the former vice chancellor, A. Gnaman; Franz P. Gruber, DZF, who presented Mohammad A. Akbarsha with a symbolic cheque to finance the building of the centre and the first year as well as an owl (the heraldic animal of the DZF); David Dewhurst, who also read greetings from the foundress H. Doerenkamp; Norbert Linke, foundation board of the DZF; Deputy Director General R. S. Sharma, a delegate of the Indian government in New Delhi; O. v. Oommen of the University of Kerala, and B. Manivannan of Panacea Biotec in New Delhi.

The formal ceremonies, symbolic laying of the cornerstone, lighting ceremony and national anthem were followed by a workshop with lectures by N. Rajendran (Mahatma Gandhi, Ahimsa and Alternative practises in Science), Franz P. Gruber (Introduction to basic concepts in alternatives), David Dewhurst (Computer-based replacement alternatives in University education – animal free teaching), S. Parthasarathy (In silico alternatives to animal use in drug discovery and development) and M. C. Sathyanarayana (Animal alternative resources).

Moving closing words and words of thanks by the new chairholder Mohammad A. Akbarsha concluded the inauguration.

The chair will have an enormous influence on the scientific scene in India and perhaps much of Southeast Asia. ALTEX aims to add to this momentum by establishing an editorial office and distribution organisation for India in Tiruchirappalli (pet named Trichy in India).

The establishment of the chair was reported on among others in the largest Indian daily newspaper, the Indian Times, and the largest newspaper in Tamil Nadu, the Hindu.

As after the establishment, the now six DZF chairs must also be kept operational, the furtherance regulations of the DZF were amended. From now on, projects submitted by the DZF chairs, and especially cooperations between the chairs, shall be favoured for funding (see also poster “The Doerenkamp-Zbinden Foundation’s chairs on alternatives to animal experimentation in research and education” in ALTEX 3/09 on page U2).

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Neural Stem Cells – Differentiation and Applications

University of Konstanz, Germany, 2 November 2009

The well-attended Mini-Symposium was organized by Suzanne Kadereit, group leader at the Doerenkamp-Zbinden Chair for Alternative in vitro Methods at the University of Konstanz.

It was opened by Mahendra Rao (Invitrogen, USA) who focused in his lecture on neural differentiation from stem cells. The field of human stem cell research holds much promise ranging from furthering our understanding of basic mechanisms of human development to the development of novel therapeutics. Rao and his group have found that human embryonic stem cell (hESC) lines tested retained pluripotent differentiation ability both in vivo and in vitro, exhibited high telomerase activity and maintained telomere length, and expressed pluripotency markers after continuous culture for at least two years. They have used these stem cell lines to generate differentiated neural populations and compared them to fetal tissue-derived and adult cells. Their data suggest that neural differentiation involves sequential and progressive stages of differentiation that can be mimicked in vitro using embryonic stem cells as a starting population. They will use dopaminergic neuron and oligodendrocyte differentiation as examples of stem cell fate choice regulation.

Lukas Sommer (University Zurich, Switzerland) discussed the area- and stage-specific growth control in neural stem cells. The formation of the vertebrate organism involves a complex process of cell division and differentiation that has to be tightly coordinated in space and time. In the nervous system, this is in part achieved by the activity of neural stem cells (NSCs) able to either self-renew or to give rise to the specialized cell types of the nervous system. Sommer and his group have recently shown that signaling pathways regulating neural stem and progenitor cell proliferation act in a brain area-specific manner, influenced by cell intrinsic local differences and by the cellular context. For instance, in the spinal cord, Wnt signal activation has a prominent effect on NSC proliferation in ventral but not dorsal regions, where Wnt is inhibited by local BMP signaling. Similarly, in the developing midbrain, but not forebrain or the adult brain, Wnt-induced stem cell expansion is counteracted by TGFβ in neural stem cells. Regulation of NSCs is not only brain area but also stage dependent. In the PNS, for instance, combinatorial Wnt/BMP signaling suppresses differentiation of early emigrating neural crest stem cells (NCSCs) and acts as a stem cell niche to maintain stem cell multipotency, while...
But can rather be subdivided into various subtypes with region and stage specific intrinsic properties and growth requirements. This highlights the necessity to establish tools by which specific NSCs types can be identified and characterized. Such knowledge will also be essential to realize the potential therapeutic use of stem cells in treating degenerative diseases or injuries.

K. Lenhard Rudolph (University Ulm, Germany) reported on telomeres, stem cells and aging. Telomeres form the ends of human chromosomes. The main function of telomeres is to cap chromosomal ends, thus, preventing chromosomal fusions and genome instability. Telomeres shorten with each round of cell division. When telomeres reach a critically short length, they lose capping function at the chromosome end and DNA damage checkpoints are induced. The activation of p53-dependent signaling pathways (including p21) induces a permanent cell cycle arrest when 4-5 dysfunctional telomeres are present in a cell. This stage is called replicative senescence. Cells can bypass the senescence checkpoint when p53 checkpoint function is abrogated.

However, further telomere shortening will induce a second checkpoint, characterized by massive chromosomal instability and cell death. This checkpoint is called crisis. Telomere shortening limits the lifespan of primary human cells (including hepatocytes) to a finite number of cell divisions. The enzyme telomerase can synthesize telomeres de novo. It consists of two essential components, the telomerase reverse transcriptase (TERT) and the telomerase RNA component (TERC) serving as a template for telomere synthesis. Telomerase is active during human embryogenesis, but the expression of TERT is lost in most somatic human tissues after birth. Only germ cells and certain stem and progenitor cells continue to express telomerase in the adult.

In humans, telomeres shorten in almost all tissues and organs during aging. In addition, there is accelerated telomere shortening in response to chronic diseases. For example, in normal human liver, there is little telomere shortening during aging. However, telomere shortening is accelerated in chronic liver disease and critically short telomeres characterize the cirrhosis stage. The telomere model of cirrhosis development indicates that telomere shortening limits hepatocyte regenerative reserve at the end stage of chronic liver disease. According to this model, fibrotic scarring occurs as a consequence of ongoing liver damage in the context of hepatocyte telomere shortening and declining regenerative reserve. The identification of new markers of telomere dysfunction and DNA damage has now provided further support to the hypothesis that the accumulation of telomere dysfunction and DNA damage contributes to human aging and the progression of chronic disease, such as liver cirrhosis.

Telomere shortening and telomerase activation also have an influence on cancer formation at multiple levels. Senescence and crisis represent tumor suppressor checkpoints that limit the proliferative capacity of transformed cells. In contrast, telomere shortening can also increase the initiation rate of tumors. Telomere dysfunction induces chromosomal instability, leading to cellular transformation. In addition, the loss in proliferative potential in aging or chronically damaged organs may select for abnormal proliferating, pre-malignant cell clones. In line with the role of telomere dysfunction in tumor initiation, the cancer risk increases in response to telomere shortening during aging and chronic diseases such as colitis ulcerosa and chronic hepatitis. Moreover, most human cancers are characterized by critically short telomeres. However, the stabilization of telomeres represents a necessary step during tumor progression. In most human cancers (including hepatic cell carcinoma, HCC), this is facilitated by activation of telomerase.

Studies in telomerase knockout mice (mTERC−/−) have provided experimental evidence that telomere shortening influences stem cell function, aging, and carcinogenesis. mTERC−/− mice with dysfunctional telomeres exhibit an impaired maintenance and function of adult stem cells correlating with impaired tissue maintenance during aging. In addition, the mice exhibit a reduced regenerative reserve in response to organ damage. In line with the dual role of telomeres in cancer formation, telomere dysfunctional mice show an increase in chromosomal instability and tumor initiation but impaired tumor progression.

Studies in double knockout mice have now provided first evidence that abrogation of DNA damage signaling pathways can improve stem cell function and organ maintenance in the context of telomere dysfunction and aging without increasing the cancer risk. Such approaches may also point to new treatment options for patients with end-stage chronic disease, such as cirrhosis.

Miriam Bibel (Novartis, Basel, Switzerland) demonstrated a cellular model system in drug discovery based on embryonic stem cell derived neurons. She and her group have devised a differentiation procedure of mouse embryonic stem cells (mESC) into homogeneous cultures of neurons in vitro (Bibel et al., Nat. Protoc., 2007; Bibel et al., Nat. Neurosci., 2004) that offers novel approaches to model neuronal processes in vitro. It enables them to work with mature neurons that in contrast to neuronal cell lines allow them to address synaptic function and neurite de- and regeneration, but also they can profit from the advantages of cell lines, such as unlimited cell culture and genetic modification.

Cell based model systems for human neurodegenerative disorders are of critical importance to assess the role of potential drug discovery targets and profile experimental therapeutics. Importantly, they have optimized conditions for long-term cultures of neurons to mature with high synaptic activity, form spines and express all isoforms of tau as neurons do in the adult brain, thus they can analyze degeneration processes of mature neurons. In particular, they introduce disease-relevant mutations, such as mutations in the proteins of huntingtin, synuclein, tau, APP, in ES cells and compare wild type and mutant differentiated neurons to analyze mechanisms of neuronal cell death and signaling pathways in neurodegenerative diseases. The homogeneity of the neuronal cultures allows them to not only successfully study functional characteristics, but also biochemical profiles and do pharmacology. The stability of the method and the unlimited cell number allows them to use the cells in primary screening.

Suzanne Kaderiet (University Konstanz, Germany) showed investigations on embryonic stem cell-derived neural cells for toxicology. Embryonic stem cells (ESC) are a promising source for reliable and reproducible cell culture systems with genetically
rather, the new legal requirements for comprehensive testing for ESC-derived cells. For instance, thousands of industrial systems could then be used for disease modeling, mechanistic generation of organ-specific cells for large-scale applications of ESC technology will likely be the generation of normal cells. While they also hold great promises for generation of cells for regenerative medicine, one of the most immediate large-scale applications of ESC technology will likely be the generation of organ-specific cells for *in vitro* assay systems. Such systems could then be used for disease modeling, mechanistic studies, drug discovery and toxicity screening. Particularly toxicity screening may become a large future application domain for ESC-derived cells. For instance, thousands of industrial chemicals in daily use have not undergone safety evaluations. Such large numbers can hardly be covered by animal-based tests. Rather, the new legal requirements for comprehensive testing will require novel cellular assays.

Kadereit showed data on differentiation of mESC to neurons and astrocytes for establishing *in vitro* assay systems to detect neurotoxicity and developmental neurotoxicity. Sub-lethal concentrations of toxicants were then tested on terminally differentiated neurons as well as on differentiating neurons. While tested glial markers and non-neural markers were not significantly affected by known neurotoxic chemicals, they observed an effect on some neuronal markers. In order to model the deleterious effects of neurotoxicants in the brain mediated by glial cells, mESC were differentiated into astrocytes. Astrocyte yield was on average 90-100%, with cells capable of inflammatory responses similar to responses from primary astrocytes. They now have the tools to develop exciting new *in vitro* systems capable of detecting neurotoxicity, developmental neurotoxicity and underlying mechanism of neurotoxicity.

The meeting was closed by Olivier Preynat-Seauve (University Geneva, Switzerland) giving an insight in pluripotent stem cell-based engineering of neural tissues to model glioblastoma development in a host nervous tissue. Pluripotent stem cells (PSC) are of interest for neurosciences since they have the potential to be differentiated *in vitro* towards cells of central nervous system (CNS). The main applications are (i) understanding of neurogenesis, (ii) engineering of *in vitro* models of CNS, and (iii) cell replacement therapy. His group reported a new method allowing neural tissue engineering in three dimensions from PSC. These tissues are a dense network of mature neural cells (neurons, astrocytes, and oligodendrocytes) able to generate and propagate electrical signals. Within this tissue, tubular structures are niches of stem cells, showing a spontaneous organization that bears resemblance to foetal brain. They are currently working on the modeling of a human brain tumor by expanding neuroblastoma cells within engineered neural tissues. The proposed model will provide a fully humanized model including a human brain tumor growing within a human host tissue.

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### Teachers call for replacement of dissections and animal experiments in life science education

Jaipur, India, 19-21 November 2009

University and college teachers attending a national alternatives conference in Jaipur, Rajasthan, India, have called for a replacement of dissections and animal experiments in life science and biomedical science curriculum. In a Resolution adopted unanimously at the "National Workshop-cum-Symposium on Potential Alternatives to Dissection and Animal Experimentation", the participants called on boards of studies at universities to remove harmful animal use from the syllabus.

"We are calling for a change in the mindset of educators, education administrators, policy makers and politicians, which should be followed by major curricular transformation in zoology and other fields where defenseless animals are used," said Brij K. Sharma, head of zoology at R. L. Saharia Government PG College, Kaladera, Jaipur. "It has been decades since the last significant change, and we encourage all boards of study to meet on a regular basis to address the pressing need for replacement of dissection and animal experiments," said Sharma, organizing secretary of the workshop, which was held from November 19 to November 21, 2009.

The national-level workshop brought together educators from the fields of zoology, anatomy, physiology and other disciplines from across Rajasthan as well as a few other states in India. It was also attended by representatives from the Government of India Agency that regulates animal experimentation, the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), which comes under Ministry of Environment and Forests.

Participants got an opportunity to learn about alternatives in life science education, get hands-on practice with innovative learning tools and share experiences. In his keynote address, Mohammad A. Akbarsha, Mahatma Gandhi-Gruber-Doerenkamp Chair (MGDC) for Alternatives in Life Science and In-Vitro Toxicology at Bharathidasan University, spoke about the evolution of the subject content of zoology – systematics, cell biology, genetics, developmental biology, physiology, ecology, evolution, etc. "Each component is no longer a simple, theoretical and/or descriptive account but has become exciting and lively with the tag of biochemistry and molecular biology added to it," he said, stressing that the emphasis on biodiversity has raised major questions about the perceived importance and relevance of dissection, which is the laboratory exercise for anatomy and the classical concepts in evolution.

"Biodiversity conservation, the need of the hour, requires that the students are taught to be kind and humane towards the animals and not treat them with contempt as dead and expendable objects. Instead of taking animals into the lab to kill, we should use alternative approaches, including respectfully studying them in the habitats where they live, without harming them," said the professor.

However, across India, thousands of animals are killed in labo-
ratories where they are used for experimentation and product testing. This is where the use of alternatives could play an important role and bring in a more humane approach. “In vitro and in silico modalities can greatly obviate this use and make learning and testing more lively and exciting,” said Mohammad A. Akbarsha, while speaking about the newly established Mahatma Gandhi-Doerenkamp Center, which also offers training programmes for educators. “Teachers will be trained in all three modalities of alternatives – computer-aided, in vitro and in silico – so that they are sensitized and motivated to change and adapt to the emerging pedagogical change in the teaching of life sciences and biomedical science,” he said.

During the event, participants also spoke about their own efforts to cut down on the use of animals in education. For instance, Chitralekha Ramachandran, speaker and trainer at the seminar, had hosted a workshop on alternatives in her college, Stella Maris in Chennai, Tamil Nadu, several years ago. “It is ironical that zoology teachers emphasize the role of every organism in the delicate balance of nature on one hand while on the other killing animals for dissection. Today, zoology teachers are experiencing an ethical crisis over animal dissection and experimentation in the laboratories,” said the teacher who has successfully implemented the study of zoology in the laboratory with the use of alternatives, thereby dropping dissection as a component in the curriculum.

“The curricular transformation is not only a challenge to the educator but is a great opportunity for reaping the benefits of every kind: scientific, economic and humanitarian,” said M. C. Sathyarayana, a trainer in the workshop and senior faculty in zoology and wildlife biology at AVC College in Mayiladuthurai, Tamil Nadu. He traced his team’s effort to reduce and even replace animal dissection through workshops and narrated how Bharathidasan University had successfully dropped dissections from their zoology curriculum. “The curriculum planners and policymakers need to drive change, taking on new and collaborative roles to integrate the emerging science of learning into the educational system. Students should be provided with the opportunity to attend dynamic, high quality curriculum designed to meet the challenges of the digital age,” said Sathyarayana.

Educators across the country, who have become more aware and sensitized, are working to make a difference and bring about a change. According to K. K. Sharma, Prof. and head of zoology at MDS University, Ajmer, the university is discouraging the use of dissection and working towards its total removal by replacement with humane alternatives. “Our future zoologists are trained not through killing but with software and models, and the use of ethical fieldwork with a conservation and biodiversity focus. It is high time that we asked what the best way to teach our students is, and considered moving from dead biology to live biology,” he said. Reena Mathur from the department of zoology at the University of Rajasthan, Jaipur, also stressed on the need to stop all dissections and use alternative techniques to teach internal morphology.

Co-ordinator of International Network for Humane Education (InterNICHE), one of the co-sponsors of the workshop-cum-symposium Nick Jukes also spoke about the widespread replacement already achieved in Gujarat and Tamil Nadu. “The momentum for curricular change is growing fast. The conference in Jaipur made clear the commitment from teachers to best practice and humane education. With international published studies so clearly demonstrating the pedagogical superiority of alternatives, I am confident that the remaining obstacles to modernization will soon fall away,” he said.

On the occasion, the MGDC, established under the auspices of Doerenkamp-Zbinden Foundation, Switzerland, gifted a copy of “The Three Rs and the Humanity Criterion”, to each of the participants. The book is an abridged version of the classic “The Principles of Humane Experimental Technique” authored by W. M. S. Russell and R. L. Burch. To support the process of replacement with alternatives, a variety of learning tools were distributed by InterNICHE-UK.

The event was organized by Brij K. Sharma, head of zoology at R. L. Saharia Government PG College, Kaladera (Jaipur). It was sponsored by the UGC, DST, InterNICHE and the International Association Against Painful Experiments on Animals (IAAPEA) and supported by the Mahatma Gandhi Doerenkamp Centre for Alternatives to the Use of Animals in Life Science Education, Bharathidasan University. The Marchig Animal Welfare Trust provided funds for the distribution of alternatives.

**Resolution**

We the participants:

1. Recognise that animals are sentient beings and that life in all its forms should be fully respected
2. Recognise the pedagogical, ethical, environmental and economic advantages of humane and innovative alternatives over animal experimentation and the dissection of purpose killed animals in life science education and training
3. Join the global movement for humane education and call for full replacement of animal experimentation and the dissection of purpose killed animals with alternatives
4. Recognise the importance of effective and ethical education and training for future Indian professionals
5. Call on the Government of India, CPCSEA, UGC, Academic Councils, Boards of Studies and educational institutes to work towards removing animal experimentation and the dissection of purpose killed animals from the life science syllabus, to develop and implement appropriate and effective laws, regulations and guidelines to bring about replacement, and to provide support for the implementation of alternatives