1st INEXO Symposium: Alternative models in vitro, ex ovo and organisms: From research to applications in pathologies and aging

Philippe Nirdé, Myriam Richaud, Hinda Dabboue, Jean-Pierre Reynier, Simon Galas, Laure-Anaïs Vincent, Jean Pierre Moles, Gilberte Marti-Mestres, Paul Chambon

Meeting Report

1st INEXO Symposium: Alternative models *in vitro*, *ex ovo* and organisms: From research to applications in pathologies and aging

doi: 10.14573/altex.1711101

The 1st INEXO Symposium was hosted on July 7, 2017 in Montpellier, France. More than a hundred scientists from eight European countries attended. Prof. Gilberte Marti-Mestres opened the first symposium expressing his hope that this would be the beginning of cycles of such symposia. Ten plenary presentations and four flash talks were given, structured into five sessions, "Cellular methods", "Reconstitution and 3D printing", "Applications", "*Ex ovo* models and chronic diseases", and "Organisms (*Caenorhabditis elegans* and tardigrades) as models". A poster exhibition was held at the end of the symposium and an award was bestowed on the best poster. Considering the current interest in alternative techniques, Prof. Paul Chambon, president of the *French Society of Pharmacy & Latina Mediterranean*, has accepted to include an INEXO symposium in the program of the XIVth Congress.

In the first session, entitled "Cellular methods", Dr **Romain Desprat** (Inst. Regenerative Medicine & Biotherapy, Montpellier, France) presented on the "Potential of induced pluripotent stem cells (iPSCs) for modeling and treating age-related human diseases". Based on recent technological innovations, it is now possible to reprogram somatic cells (usually peripheral blood monocytes) into iPSC. He applied this technology to generate cellular models of three helicase-linked early aging syndromes. He showed that the cells reflected the cellular-related physiology and cellular senescence after their differentiation into mesenchymal stem cells and their potential derivatives. The iPSC lines will allow the study of the cellular and molecular mechanisms linked to senescence and genomic instability pathologies of premature aging syndromes for the first time and can be used to screen for potential therapeutic compounds to correct the cellular dysfunction.

Prof. **Catarina Brito** (Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal) explained that the demand for robust and predictive human *in vitro* models is steadily increasing. Neural cell fate and functionality are highly regulated processes in which the microenvironment plays a crucial role. Alterations of such processes can lead to abnormal neural development and function and to degeneration. She uses perfusion stirred-tank bioreactors to achieve 3D neural differentiation. This strategy induces neural progenitor cell aggregation and subsequent differentiation into complex tissue-like structures with reproducible ratios of neurons, astrocytes and oligodendrocytes. The generated neurons elicit spontaneous calcium transients and stimuli-induced neurotransmitter release. Whole-cell current-and-voltage clamp recordings show polarized neurons and voltage-dependent ion currents. Differentiated glial cells present astrocytic functions. Moreover, expression of genes involved in synaptic and ion transport machinery and the accumulation of neural proteoglycans suggests that this 3D differentiation strategy mimics the neural tissue microenvironment better than other differentiation methods. These models have applications as tools for preclinical assessment and in disease modelling.

In the next session on reconstituted tissues and 3D bioprinting, Dr **Christian Pellevoisin** (Episkin Academy, Lyon, France) spoke about reconstructed skin, which is a powerful and highly versatile technology already used at all stages of cosmetic product development (toxicology, UV sensitivity, skin allergy, skin aging, skin microbiome, etc). The ability to reproduce several functions of human skin *in vitro* broadens the scope for industrial applications. He demonstrated that it is now possible to predict positive or negative effects of cosmetics early in their development process using *in vitro* skin models instead of animal testing. Reconstructed human skin is also used for screening and assessing the efficacy of new active ingredients, deciphering their mechanism of action, and optimizing the composition of formulations.

Prof. **Mikael Garcia** (Poitis, Pessac, France) presented a talk on 3D printing. Dealing with tissue complexity and reproducing the functional anisotropy of human tissues remains a challenge for tissue engineers. Experimental data showing that cell fate is controlled by biochemical and/or mechanical cues arising from the cell microenvironment suggests that tissue formation obeys short range orders without reference to a macroscopic or global pattern. In that context, an improved tissue engineering strategy might rely on guiding tissue morphogenesis from the cell to the tissue level. Laser-assisted bioprinting (LAB) applications so far have been limited to biofabrication of thin constructs. Dr Garcia now presented an original 3D multimodal and modular bioprinter that combines LAB with microvalve bioprinting.

Cells can be printed at cell resolution using LAB while the biomaterials are printed at a coarser resolution (100 µm) using microvalve bioprinting. One mm thick 3D constructs can be printed with different biomaterial layer thicknesses and with multiple cell micropatterns across tissue constructs. Combining technologies featuring different resolutions opens new horizons for controlling micro- and macro-organization of tissue components, and hence for guiding cellular morphogenesis within thick 3D tissues.

In the session on "Applications", Dr **Laurent Lamy** (Nestlé Skin Health – Galderma R&D, Sophia-Antipolis, France) and his team introduced the cellular thermal shift assay (CETSA®), which facilitates the direct assessment of target engagement within cells and tissues at various stages of drug development. The CETSA® method builds on the concept of thermal stabilization of target proteins upon ligand binding in biological samples. By quantifying the melting temperature and shift induced by the ligand, the potency of target engagement can be quantified. For detecting thermodynamic stability using CETSA®, drug- or vehicle treated cell lysates or intact cells were heated to different temperatures and the target proteins also were detected by Western blotting. The data demonstrates that CETSA® can assess drug target engagement in intact cells and thus replace the low-throughput Western blot with a quantitative, higher throughput assay. CETSA® is likely to be applied in many stages of drug development from high-throughput screening assays to clinical trials.

Prof. **Ana-Maria Fadda** (University Cagliari, Italy) explained that *in vitro* techniques are used extensively in academia and industry to assess skin drug penetration and permeation because they predict human dermal penetration well, provide results quickly, and are time- and cost-saving. Static test cells are composed of donor and receiver compartments of different size and a diffusional surface. Excised skin specimens are sandwiched as a barrier between the two compartments and the formulation is applied to the skin surface. The receiver contains a fluid that simulates the blood flow; it should closely simulate *in vivo* conditions of permeation and guarantee sink conditions. Alcohol, albumin or cyclodextrin are added to the drug if its water solubility is low. Drug permeating from the donor to the receiver compartment is determined as a function of time by receptor fluid removal from the sampling port at regular intervals. Flow-through cells can be useful when the permeant has very low solubility in the receptor medium. A potential disadvantage of the *in vitro* studies is the lack of information regarding effects of blood flow on drug permeation.

Prof. *Domenico Ribatti* (Department of Basic Medical Sciences, University of Bari Medical School, Italy) started off the session on "*Ex ovo* models and chronic diseases" with his lecture on the chick embryo chorioallantoic membrane (CAM). The CAM is an extraembryonic membrane which serves as a gas exchange surface and its function is supported by a dense capillary network. It has been broadly used to study the morphofunctional aspects of angiogenesis *in vivo* and to investigate the efficacy and mechanisms of action of pro-angiogenic and anti-angiogenic natural and synthetic molecules. He presented the use of the CAM in the context of tumor angiogenesis and metastasis studied during the chick immune incompetent period. New blood vessels penetrated the tumor after 72 h implantation and favored its rapid growth. The CAM is also used to study tumor invasion of the epithelium and the mesenchymal connective tissue, where it forms a dense bed of blood vessels.

Dr **Pascal De Santa Barbara** (Inserm, Montpellier, France) spoke on "Avian retroviral transgenesis approaches: gain & loss of function". The embryonic chick provides an excellent model system for studies of developmental biology. The model is used to target the digestive musculature layer after injection of avian retrovirus in an early stage of egg development. Replication competent avian leukokosis virus long terminal repeat with a splice acceptor (RCAS) is a replication-competent retroviral vector system that allows *in ovo* sustained misexpression of a gene of interest. RCAS is a modified version of an avian Rous sarcoma virus and has been used in gain- and loss-of-function approaches to identify key signaling pathways and factors involved in organ and tissue development. Advantages and limitations of the RCAS approaches were discussed.

The last session entitled "Organism models" started with Dr **Florence Solari** (Institute NeuroMyoGene, CNRS UMR5310, Inserm U1217, Université Claude Bernard, Villeurbanne, France), who presented the role of the transcription factor UNC-120/SRF in aging and lifespan of *C. elegans*. Since muscle deterioration compromises life quality during aging, the time course of muscle aging at the subcellular and physiological levels in *C. elegans* was analysed. A dramatic decrease in the expression of genes encoding proteins required for muscle contraction, followed by a change in mitochondria morphology, and an impairment of muscular autophagy was observed. It could be demonstrated that the conserved transcription factor UNC-120/SRF controls muscle aging biomarkers independently from its effect on lifespan. In daf-2/insulin/IGF1 receptor mutants, which exhibit a delayed appearance of muscle aging biomarkers and are long-lived, disruption of unc-120 accelerated muscle aging but did not shorten lifespan extension. Overall, the study identified UNC-120/SRF as the first transcription factor that controls the pace of muscle aging in a cell autonomous manner.

Prof. **Michael Wink** (Heidelberg University, Institute of Pharmacy and Molecular Biotechnology, Heidelberg, Germany) explained more about the *C. elegans* model. This nematode shares a high number of genes and regulatory pathways that are relevant in the context of diseases and health disorders with humans. It is easy to keep hundreds and thousands of nematodes with a lifespan of 2-3 weeks. They are ideal systems to study aging and the effect of drugs on this process. A large number of mutants have been developed, which are adapted to disease conditions, such as mutants that express the Alzheimer protein a-beta or elements of oxidative stress. Several mutant or transgenic strains have been made in which relevant key enzymes or proteins are tagged with GFP, which facilitates the analysis of drug activity. Prof. Wink has studied the effects of several medicinal plants, nutraceuticals and isolated plant secondary metabolites in *C. elegans* focusing

on finding compounds that exhibit antioxidant and antiaging properties and also works on aspects of neurodegenerative diseases.

The flash talk session was opened by Dr **Nicolas Lebonvallet** (Laboratoire Intéractions Epitheliums-Neurones, Brest, France). His aim was to directly differentiate stem cells derived from the neural crest into sensory neurons. He used skin-derived precursor (SKP) cells derived from the neural crest and extracted from the skin tissue by enzymatic and mechanical dissociation. These cells were used to induce the Wnt and the BMP pathways. After differentiation, they acquired a sensory neuron phenotype exhibiting a bipolar neuronal morphology and expressed neuronal markers (Brn3a, p75NTR and peripherin, TRPV1 channel).

Dr **Josep-Lluís Viladot** (Lipotec, S.A.U., Barcelona, Spain) communicated on the transference of actives from polymeric film (typically used for face masks and eye patches) to skin by means of a percutaneous absorption test with porcine skin biopsies. He showed that it is possible to quantify the amount of active compound that is present in every skin layer by extraction with selective solvents and HPLC analysis. By carrying out testing after different incubation times, the kinetic pattern allowed a certain prediction of *in vivo* results and the possibility of screening different candidates.

Dr **Amanda Finan-Marchi** (Inserm U1046 – CHU, Montpellier, France) used the CAM to study *ex vivo* cardiac function. She grafted dissociated cardiomyocytes or pieces of avian (allograft) or mammalian (xenograft) heart fragments that were then vascularized by the CAM. The grafts regained functionality as evidenced by beating, and stimulation by epinephrine significantly enhanced the beat rate frequency of the grafted tissues. The results demonstrate that cardiac grafts find a complex and supportive environment (*ex ovo*) to recover functional properties. This work provides a novel method to extend *ex vivo* studies of cardiac function.

Prof. **Lorena Rebecchi** (Università di Modena e Reggio Emilia, Dipartimento di Scienze della Vita, Modena, Italy) presented an emerging alternative model organism for biomedical research, the tardigrade (water bear) that survives freezing, high temperatures, irradiation, vacuum of outer space, and complete desiccation (losing 97% of body water) owing to reversible suspension of its metabolism called anhydrobiosis. This involves a DNA –associating protein (Dsup) and specific intrinsically disordered proteins (TDPs). The next challenge will be to induce or engineer complete desiccation tolerance in cells/tissues of desiccation sensitive organisms, using the xeroprotectants detected in tardigrades.

We thank all speakers for their contributions to the success of the symposium.

Philippe Nirdé[1], Myriam Richaud[1], Hinda Dabboue[1], Jean-Pierre Reynier[2], Simon Galas[1], Laure-Anaïs Vincent[1], Jean-Pierre Moles[3], Gilberte Marti-Mestres[1] and Paul Chambon[4]

[1]Institut des Biomolécules Max Mousseron (IBMM), CNRS UMR 5247, Université de Montpellier, ENSCM, Montpellier, France; [2]Assistance Publique Hopitaux de Marseille (AP-HM), Marseille, France; [3]INSERM U1058, Université de Montpellier, Montpellier, France; [4]Société Française de Pharmacie de la Méditerrannée Latine, Saint Genis-Laval, France