Cellular methods
Potential of Induced Pluripotent Stem Cells (iPSCs) for modeling and treating age-related human diseases

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SUMMARY:
Helicases are enzymes involved in the replication, transcription and repair of DNA. Their deficiency is responsible for clinically identified syndromes: Bloom syndromes, Werner and Rothmund-Thompson syndrome. All are characterized by a strong predisposition to cancer and by a phenotype of premature aging of the affected persons. If the genes involved in these pathologies are identified, little is known about the mechanisms responsible for cellular senescence and genome instability. For these 3 pathologies, no therapeutic, preventive or curative solution has been proposed.

Because animal models and differentiated cell studies are incomplete, the study of stem cells in these patients is essential. Recent technological innovations make it possible to reprogram somatic cells into induced pluripotent cells (iPS): this is an approach of choice to study the pathophysiology of a syndrome, to obtain differentiated cells of for disease recapitulation and to carry out the screening of Pharmacological active ingredients.

Our project is to generate iPS lines of 3 helicase-linked early aging syndromes from PBMC. We will study recapitulation of cellular related physiology and cellular senescence after their differentiation into Mesenchymal stem cells and their potential derivatives.

This project will enable us to create for the first time iPS cell lines for the pathologies of premature aging linked to helicases. Through these, it will be possible to study directly the cellular and molecular mechanisms, in particular the senescence and the genomic instability. The availability of these lines makes it possible to envisage the screening of therapeutic compounds which can correct the dysfunction of the cells.

REFERENCES:


Exploring human Stem Cells to mimic neural microenvironment

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SUMMARY:
The demand for robust and predictable human in vitro models that can bridge the gap between preclinical and clinical stages of drug discovery, and contribute to increase the understanding of human diseases, is steadily increasing. Neural fate and functionality are highly regulated processes that integrate a wide range of external cues, such as nutritional status, growth factors, mechanical stress, cell-cell and cell-extracellular matrix (ECM) interactions. Indeed, brain microenvironment plays an important role in neurodevelopment, function and degeneration.

We employ perfusion stirred-tank bioreactors for 3D neural differentiation of an array of human stem cell sources. 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. Under whole-cell current-and-voltage clamp, recordings showed polarized neurons and voltage-dependent ionic currents. Differentiated glial cells presented astrocytic functions, such as glutamate clearance and glutamine synthesis. Moreover, gene expression of synaptic and ion transport machinery, as well as accumulation of neural proteoglycans suggests that this 3D differentiation strategy better mimics neural tissue microenvironment than other differentiation methods currently available. Applications of these models as tools for preclinical assessment and in disease modelling will be discussed.

ILLUSTRATION:

KEYWORDS:
3D cell models, human stem cells, extracellular matrix, perfusion stirred-tank bioreactors, neural differentiation

REFERENCES
Reconstitution
& 3D printing tissue
**SUMMARY:**

Skin engineering is a powerful and highly versatile technology used at all stages of a cosmetic product development. The contributions of reconstructed skin are significant in many areas of research, for example: 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. Thanks to in vitro skin models it is now possible to predict, early in their development process, some positive or negative effects of cosmetics without the need of animal testing. Reconstructed epidermis and skin tissues, represent a potential strong driver for the development of in vitro methods to face this new paradigm in toxicology. To date, several in vitro methods have been developed to assess different toxicological endpoints, some of which have been validated and recognized by the regulatory bodies concerned. Reconstructed human skin is also an unbeatable tool for screening and assessing the efficacy of new active ingredients, deciphering their mechanism of action and finally, to optimize the composition of formulations to maximize in vivo benefits.

**KEYWORDS:**

- Reconstructed human epidermis
- efficacy testing
- Immunology
- reconstructed human skin
- alternative to animal methods
- Microbiome
- toxicology
- T-Skin
- RHE

**ILLUSTRATION:**

EPISKIN model

Production of the full thickness T-Skin model

SkinEthic RHE colonized with CD4+ T cells
Dealing with tissue complexity and reproducing the functional anisotropy of human tissues remain a puzzling challenge for tissue engineers. Emergence of the biological functions results from dynamic interactions between cells, and with extracellular matrix. Experimental data showing that cell fate (migration, polarization, proliferation…) is triggered by biochemical and/or mechanical cues arising from cell micro-environment suggests that tissue formation obeys to short range orders without reference to a macroscopic or global pattern. In that context, the winning tissue engineering strategy might rely on guiding tissue morphogenesis from the cell to the tissue level.

From a technological point of view, the Laser-Assisted Bioprinting (LAB) technology has emerged as an alternative method to inkjet and bioextrusion methods, thereby overcoming some of their limitations (namely clogging of print heads or capillaries) to pattern living cells and biomaterials with a micron-scale resolution and high cell viability. LAB applications has been limited so far to biofabrication of thin constructs.

In this work, we present an original 3D multimodal and modular bioprinter which combines LAB with microvalve bioprinting. Thanks to this system, cells can be printed at cell resolution using LAB while biomaterials are printed with a coarser resolution (100 µm) using microvalve bioprinting. Interestingly, we show that 1 mm thick 3D constructs can be printed with different biomaterial layer thicknesses (eg made of collagen, agarose) and with multiple cell micropatterns across tissue constructs.

In conclusion, combining technologies featured by different resolution opens new horizons for controlling micro and macro organization of tissue components, and hence for guiding cellular morphogenesis within thick 3D tissues.
Applications
CETSA®: a target engagement assay to improve drug
discovery efficiency

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SUMMARY:
A drug must engage its intended target to achieve the therapeutic effect. Proof of drug-target engagement in
physiologically-relevant contexts is a key pillar of successful therapeutic target validation. Several key analyses of
the causes of clinical trial failures were recently published. In as many as half of the programs that failed due to ‘lack of
efficacy’, a lack of demonstration of the intended target was identified. To better understand why some drugs, despite
being potent inhibitors in biochemical assays, lose in vitro or in vivo activity, it is necessary to determine to what extent
drugs engage their primary targets.

However, conclusively measuring target engagement in situ is challenging. This point is critical for topical drugs due to
barrier function of the skin: “The reason for being” of the Epidermis. There are few technologies that enable the
accurate measurement of drug-target occupancy in physiological contexts. In the present project we aimed to meet
this challenge by developing a label-free, biophysical assay: the cellular thermal shift assay (CETSA®), which
facilitates the direct assessment of target engagement in 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 cells and
tissue samples. By quantifying the melting temperature and shift induced by the ligand we can quantify the potency
of target engagement. This potency determination then allows filtering of drug candidates by their ability to engage the
target in its physiologically relevant form. For detecting thermodynamic stability using CETSA®, drug- or vehicle-
treated cell lysates/intact cells were heated to different temperatures and the target proteins were detected by western
blotting.

Our data demonstrates that CETSA® can assess drug target engagement in intact cells. These initial successes
prompt us to develop alternative strategy to replace the low-throughput, manually intensive Western blot readout, with
a quantitative, higher throughput assay. This would provide sufficient capacity to utilize CETSA® as a primary hit
qualification strategy. We are also confident that this assay will prove to be a valuable tool to allow the direct
confirmation of cellular target engagement in an ex-vivo human skin model, supporting clinical application.

CETSA® is likely to be a relevant tool applicable in many stages of drug development from High-throughput screening
assays to clinical trials.
Pharmaceutical applications: \textit{in vitro} using diffusion cells

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**SUMMARY:**

Academia and industry have been used extensively \textit{in vitro} techniques to assess skin drug penetration and permeation because they are appropriate to predict human dermal penetration, give results quickly, are time- and cost-saving, and generally show better reproducibility of results. Moreover, these experiments can be performed using either human or other mammalian skin samples. However, the experiments should be performed following the "OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption in vitro method"

The most common methods for evaluating \textit{in vitro} skin penetration employ diffusion cells, and a rich literature confirms the suitable performance of these experiments. A potential disadvantage of the \textit{in vitro} studies is the lack of information regarding effects of blood flow on drug permeation, since the \textit{in vivo} sink conditions cannot be completely reproduced.

Diffusion cell design may vary from a simple two compartment "static" or a more complex "flow-through" system. The static cells are composed of two compartments, the donor and the receiver, and are usually vertical (Franz cells) or side-by-side. They can vary in size, and diffusional surface.

Excised skin specimens or are sandwiched as a barrier between the two compartments, with the SC side facing the donor compartment and the formulation is applied on the skin surface. The receiver contains an appropriate fluid that simulates the blood flow and is continuously mixed by a stir bar. The ideal receiver fluid should well simulate the \textit{in vivo} situation of permeation and guarantee sink conditions. Indeed, it is generally recognised that if the drug water solubility is less than 10 µg/ml, the water receiver fluid must be added of solubilizers such as alcohol, albumin, or cyclodextrin. The receiver fluid must be thermostated to ensure that the skin surface temperature is kept at the \textit{in vivo} conditions (32±1°C). The drug permeating from the donor to the receiver is determined as a function of time by receptor fluid removal from the sampling port at regular intervals. To ensure sink conditions, the removed solution must be replaced with an equivalent amount of fresh receptor fluid. A rich literature regarding diffusion cell design and use is available.

Flow-through cells can be useful when the permeant has very low solubility in the receptor medium.

A potential disadvantage of the \textit{in vitro} studies is the lack of information regarding effects of blood flow on drug permeation, since the \textit{in vivo} sink conditions cannot be completely reproduced.
Ex-ovo models (CAM) & Chronic diseases
The chick embryo choriollantoic membrane in the study of tumor angiogenesis

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SUMMARY:
The chick embryo chorioallantoic membrane (CAM) is an extraembryonic membrane which serves as a gas exchange surface and its function is supported by a dense capillary network. Because of its extensive vascularization and easy accessibility, the CAM has been broadly used to study the morpho-functional aspects of the angiogenesis process in vivo and to investigate the efficacy and mechanisms of action of pro-angiogenic and anti-angiogenic natural and synthetic molecules. The CAM is a favored system for the study of tumor angiogenesis and metastasis, because at this stage the chick immunocompetence system is not fully developed and the conditions for rejection have not been established. Tumors remain avascular for 72 hr, after which they are penetrated by new blood vessels and begin a phase of rapid growth. The rate of growth during this vascular phase is greater for implants on days 5-6, and decreases for later days of implantation. Other studies using the tumor cells/CAM model have focused on the invasion of the chorionic epithelium and the blood vessels by tumor cells. The cells invade the epithelium and the mesenchymal connective tissue below, where they are found in the form of a dense bed of blood vessels, which is a target for intravasation.

ILLUSTRATION:

Example of tumor xenograft on the CAM

Immunohistological analysis showing blood vessels
(From Ribatti et al. Br J. Cancer)
Avian retroviral transgenesis approaches: gain & loss of function

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SUMMARY:
The embryonic chick provides an excellent model system for studies of developmental biology. RCAS (Replication Competent ALV LTR with a Splice acceptor) is a replication-competent retroviral vector system that allows in ovo sustained misexpression of a gene of interest in avian cells. RCAS is a modified version of an avian Rous sarcoma virus and this tool has been used to gain- and loss-of-function approaches to identify key signaling pathways and factors involved into organ and tissular development. Moreover these approaches are now widely used in cell biology. Advantages and limitations of the RCAS approaches will be discussed.

KEYWORDS: chick model  in ovo  transgenesis  morphogenesis

REFERENCES
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Organisms as models
(C. elegans, tardigrades, etc....)
UNC-120/SRF independently controls muscle aging and lifespan in C. elegans

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SUMMARY:
Lifespan is the main read-out used in model organisms for aging studies but the relationship between longevity and health span is still a matter of intense debate. Since muscle deterioration compromises life quality during aging, we analyzed the time course of muscle aging at the subcellular and physiological levels in C. elegans. We first observed 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. We 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 accelerates muscle aging but does not shorten lifespan extension. Overall our study identified UNC-120/SRF as the first transcription factor that controls the pace of muscle aging in a cell autonomous manner.

ILLUSTRATION:

KEYWORDS:
C.elegans Aging Muscle SRF/UNC-120 DAF-2/insulin-IGF-1 Rc

REFERENCES
Unpublished results
**C. elegans - a powerful model system for drug development**

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**SUMMARY:**

The nematode *C. elegans* shares a high number of genes and regulatory pathways with humans which are relevant in the context of diseases and health disorders. It is easy to keep hundred and thousands of these nematodes which only live 2-3 weeks. Therefore, they are ideal systems to study aging and the effect of drugs on this process. Furthermore, a large number of mutants have been developed, which are adapted to disease conditions, such as nematodes, which express the Alzheimer protein a-beta or elements of oxidative stress. In many of these mutants, relevant key enzymes or proteins are tagged with GFP, which facilitates the analysis of drug activities. We have studied the effects of several medicinal plants, nutraceuticals and isolated plant secondary metabolites in *C. elegans*. Our focus was to find compounds, which exhibit antioxidant and antiaging properties. Another emphasis is on neurodegenerative diseases.

**ILLUSTRATION:**