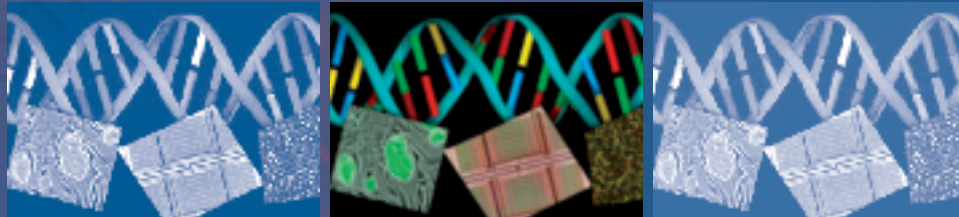pagepress

biomedical science
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The role of 3Rs in the age of One Health:
where we are and where we're going

September 13-15, 2023

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September 13-15, 2023

UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA
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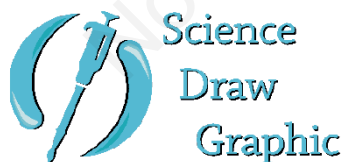
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toxin MPTP and the clearance inhibitor probenecid (MPTPp), by combining *in vivo* PET imaging and immunohistochemistry.

A group of 10 mice were injected with 100 mg/kg of probenecid followed by 25 mg/kg of MPTP, twice a week, for a total of 5 weeks. They were monitored longitudinally with PET before treatment and after 1, 3 and 10 MPTPp injections using two radiotracers: [18F]-FP-CIT, a marker of Dopamine Transporter (DAT) and [18F]-FDG to assess brain glucose metabolism. They were then sacrificed and brains collected for post-mortem immunohistochemical analysis.

We found that both striatal DAT-binding in-vivo assessed with [18F]-FP-CIT PET and the density of striatal DAT-positive fibers observed post-mortem started to decrease significantly after 3 MPTPp injections. [18F]-FDG uptake was significantly decreased in the striatum and thalamus already at the first administration, while at 10 MPTPp injections [18F]-FDG uptake was increased in the somatosensory and somatomotor cortex. Our results suggest that glucose metabolism is an earlier marker than DAT-binding in detecting neurodegeneration.

The fantastic voyage of solid lipid nanoparticles from the lung to the brain: non-invasive tomographic imaging as a feasible refinement process

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Solid Lipid Nanoparticles (SLN) are colloidal drug delivery systems characterized by higher entrapment efficiency, good scalability of the preparation process and increased sustained release of the payload. Surface functionalization of SLN with ligands to achieve a site specific targeting makes them attractive to overcome the limited Blood-Brain Barrier (BBB) penetration of therapeutic compounds. SLN are prepared for brain targeting by exploiting the adaptability of warm microemulsion process for the covalent surface modification with an Apolipoprotein E-derived peptide (SLN-mApoE). Furthermore, the influence of the administration route on SLN-mApoE brain bioavailability is here evaluated by means of Fluorescence Molecular Tomography, an advanced optical imaging technology that uses the Near-Infrared Spectrum (NIR) (600–900 nm) for non-invasive *in vivo* imaging and Three-Dimensional (3D) quantification of the fluorescent probes. Fluorescent labelled SLN-mApoE are able to cross intact a BBB *in vitro* model. The pulmonary administration of SLN-mApoE is related to a higher confinement in the brain of Balb/c mice compared to the intravenous and intraperitoneal administration routes, without inducing any acute inflammatory reaction in the lungs. These results promote the pulmonary administration of brain-targeted SLN as a feasible strategy for improving brain delivery of therapeutics as well as the FMT's ability of quantitative assessment *in vivo*-bio-distribution studies.

Nebuloid: a novel *in silico* agent-based cell model

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Proliferation and resource consumption of cells are predicted using a classic continuum approach in *in silico* models. For instance, in a Three-Dimensional (3D) cell-laden spheroid consuming oxygen, the construct is represented as a unique finite domain through which oxygen flux is governed by the diffusion and consumption equation. Although this approach is widely used for several applications, it has some limitations. As a matter of fact, encapsulated cells in 3D structures are composed of discrete consuming units within extracellular non consuming space. Thus, *in silico* models assume consumption in all the nodes of the mesh (*i.e.*, the domain where the physics applies) using an estimated cell density. Moreover, they do not take into account the real arrangement of the cells within the construct or consider any regions occupied by extracellular matrix and do not attribute cell-specific metabolic parameters, which do in fact change with phenotype. Here, we propose an *in silico* model developed with the COMSOL (COMSOL Inc., Stockholm, Sweden) Livelink environment for Matlab, where cells within the construct were modelled as a point cloud with a homogeneous spatial distribution. In the simplest model, the cells consume oxygen following the Michaelis-Menten equation, with the same metabolic parameters (sOCR and Km). The metabolic rate (B) was calculated as the inward flux at spheroid surface for spheres with different radius and same cell density ($5.14e12$ [cell/m³]). Preliminary results show discrepancies in the values of B between the bulk continuum model and the one obtained in the Nebuloid model developed here. Nebuloid allows control of the spatial position and the metabolic parameters for each cell: this is crucial for developing more relevant and predictive models for 3R approaches.

From data exploration to predictive models: advanced Machine Learning and Artificial Intelligence techniques for cardiotoxicity analysis

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In recent years, cardiovascular toxicity has attracted considerable attention from scientists and clinicians since Cardiovascular Disease (CVD) is one of the leading causes of mortality worldwide. However, except for drugs, the evaluation of the potential cardiotoxic effects of chemicals is poorly addressed and regulated. *In silico* methodologies are rapidly emerging as an essential tool in toxicology and pharmaceutical research. These approaches comprise a series of methodologies, which can play an important role in the reduction,

the molecular mechanisms involved in SSc pathogenesis. Moreover, this model represents an innovative approach offering the possibility to interconnect different cell types in a dynamic environment and to reproduce *in vitro* and *in vivo* tissue/organ architecture. Further applications will consist in investigating the pathogenic mechanisms of several diseases, testing new drugs, employing stem cells to build the organs for transplantation through a model resembling the *in vivo milieu* with a significant focus on the ethical animal handling.

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Development of personalized preclinical models for drug screening in Chronic Lymphocytic Leukemia using 3D bioprinting

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Preclinical models employed for haematological cancer research are mainly Two-Dimensional (2D) *in vitro* culture and animal models. However, they present many limitations that can be potentially overcome by Three-Dimensional (3D) cell culture. The aim of the project is to employ 3D bioprinting to generate personalized preclinical models to study the response to therapies in Chronic Lymphocytic Leukemia (CLL), the most common adult leukemia in the western world, that remains incurable. MEC1 CLL cell line and primary cells were bioprinted within different hydrogels supporting cell viability and treated with chemotherapy for 24 and 72h at time zero post printing or after 7 days of 3D culture adaptation. RNAseq analysis on 3D bioprinted primary cells after 7 days of culture show that cells compared to 2D cultured overexpress genes involved in proliferation, survival and homing within lymphoid tissues. Prompted by these results we employed 3D bioprinted CLL cells, to evaluate their preclinical application for drug testing. We firstly observed an increased resistance to the drug in MEC1 cells after 7 days of adaptation in static culture compared to 2D. In addition, dynamic culture settings were found to further influence cell response in 3D. Similarly, we observed that 3D bioprinted primary cells show a higher resistance to chemotherapy if compared to 2D, after 72h treatment. We could achieve comparable response by increasing the dose of drug however, by treating the primary cells after 7 days adaptation in 3D they became resistant at both doses. By rtPCR we demonstrated that genes identified by RNAseq and potentially involved in resistance to therapy are differentially expressed in 3D settings compared to 2D culture. We are implementing the co-printing with other cells types mimicking the lymphoid environment to evaluate the contribution of the microenvironment in response to drugs. These results pave the way for the generation of more complex *in vitro* models to assess the response to target therapies in a personalized manner.

P28

Designing 3D bioprinted meniscal scaffold taking inspiration from Extracellular Matrix (ECM) features

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The menisci are C-shaped cushions found in the knee articulation between the femoral condyles and the tibial plateau which main function is the weight distribution. Their Extracellular Matrix (ECM) is highly hydrated, and mainly composed of collagen, Glycosaminoglycans (GAGs), adhesion glycoproteins and elastin. The meniscus is composed by two portions, the inner and the outer, with different composition, fibres orientation and properties. The meniscal tissue has a low vascularization and low regeneration potential; on the other hand, injuries are very common.
Here in this work, the generation of Three-Dimensional (3D) bioprinted meniscal tissue is proposed. To mimic the ECM morphology of meniscal tissue, ECM has been characterized by Scanning Electron Microscope (SEM) analysis. Furthermore, major ECM components have been characterized and compared. The mechanical properties of the tissue were then analysed with a Finite Element (FE) simulation on models obtained from the segmentation of DICOM files from nuclear magnetic resonance on patients.
A 3D bioprinted meniscal scaffold is proposed and to mimic the inner and outer inner and the outer parts of the meniscus two bioprintable ECM mimetics have been produced and characterized.

P29

InExpose and vivoFlow system: advances in Refinement and Reduction

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In last decades, scientific research is doing its utmost to implement and achieve the ethical principles of the 3Rs. In this respect, our state-of-the-art platform inExpose system (SCIreQ; Emka Technologies, Sterling, USA) is an example. The latter is a compact exposure system of precision inhalation in nose-only mode, whose computer-controlled nebulizers allow automated generation of aerosols with a precise concentration in the order of seconds. Moreover, our modular platform is combined with vivoFlow system (SCIreQ, Emka Technologies), that allows a whole-body mode inhalation and a contemporary recording of rodents plethysmo-

graphic values. Our combined platform offers the great opportunity to performing small-scale studies, evaluating small animal numbers in precise controlled conditions. Thus, this system allows us to study nanoparticles impacts on murine models along repeatable and non-invasive exposures. Moreover, we have the possibility not only of minimising any animal distress but also of monitoring the animal welfare during the entire exposure procedures assuring with a better reliability and repeatability of scientific results. In conclusion, this innovative nanotechnological platform is open to the contribution of research groups united by the aim of identifying the toxicological profile of inhaled nanoparticles and validating the development and refinement of experimental models to study the efficacy and safety of the inhalation administration route of new drugs.

P30

3D glioblastoma *in vitro* models to identify the impact of ECM in tumor progression

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The Extracellular Matrix (ECM) is a dynamical microenvironment where aberrant balance of proteins, glycoproteins, Glycosaminoglycans (GAGs) and Hyaluronic Acid (HA) favor tumor progression and invasiveness.

The generation of tailorable, *in vitro* systems able to replicate physical and biochemical features of ECMs will contribute to spreading light on the role of the cell microenvironment features determining the pathological event and will allow to develop animal-free drug testing protocols.

In this work, we aim to understand and test the effect of biochemical and physical behavior in Glioblastoma Multiforme (GBM) microenvironment and display how *in vitro* systems candidate as forerunners for future biomedical studies. To this end, HA was crosslinked with different ECM proteins, exploiting linkers with different lengths and branching. The selected formulations were first tested with three cell lines to obtain an *in vitro* 3D bioprinted GBM model suitable for high-performance, predictive screening. Next, their dynamical properties were investigated by applying a flow rate. Finally, since cells respond to a variety of *stimuli*, we have also synthesized and functionalized a glycoconjugate hydrogel to understand the effect of glucose on GBM proliferation and invasion. The outcome of these three studies was the preparation of neurospheres inside the hydrogel, which we ultimately tested for different drugs for cross-validation.

P31

The prolonged effects of Russian chrysotile on an *in vitro* 3D human lung epithelial tissue

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Asbestos fibres, including the amphiboles and the serpentine Chrysotile (CHR), are classified as carcinogens by the International Agency for Research on Cancer (IARC). Today CHR "safe" mining takes place only in few countries like Russia, China, Africa, and South America, although, to mitigate the health risks associated with CHR, various international organizations, including the World Health Organization (WHO), have advocated for a complete ban on asbestos.

This study investigated the effects of a Russian Chrysotile (CHR) from the Yasny mine, on a Three-Dimensional (3D) human lung organotypic *in vitro* model: the EpiAirway™ (MatTek Corp, MA, USA). This model was used to investigate the 12-days effects of CHR, physically separated in two fractions of different fibre lengths (<5 and >5 μm) on viability, barrier integrity, and inflammation. Results were compared to the effects of Crocidolite (CRO) fibres considered as a carcinogenic positive control. The results showed that tissue viability was significantly reduced for both CHR fractions and CRO at 24 h, although at 12 days the viability returned to the values of the untreated control in all samples, indicating a significant resilience of the tissue in the long term, also confirmed by the morphological analyses and the Transepithelial Electrical Resistance (TEER) measurements. Gene expression analyses at 24 h revealed an increase in IL-1β, IL-6 and IL-8 pro-inflammatory cytokines, while ELISA tests showed a significant release of IL-1β, TNFα and IL-8 at 24 and 48h. Conversely, at 12-days of fibre exposure, only CRO and the CHR largest fraction induced an increase of IL-1β, and IL-8 gene expression compared to the untreated control. This preliminary study gives new insights into the role of fibre length in the toxicity of minerals and suggest the possibility to use the physiologically more relevant 3D lung tissue models to study the long-term effects of mineral fibres with respect to the traditional Two-Dimensional (2D) lung cellular models.

P32

Novel three-dimensional *in vitro* models of endometriosis

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