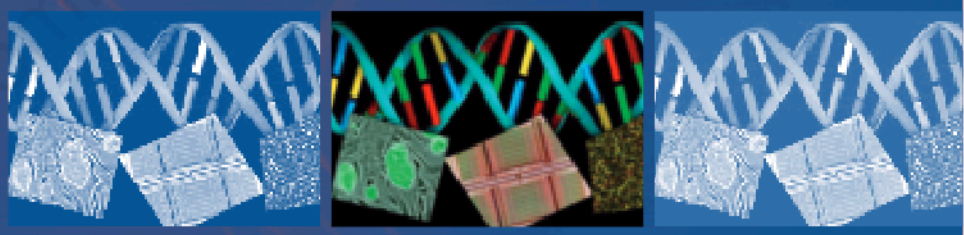
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The role of 3Rs in the age of One Health: where we are and where we're going

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Editor-in-Chief

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Valeria Chiono is Full Professor at the Department of Mechanical and Aerospace Engineering of Politecnico di Torino, Italy. She is lecturer of "Engineering for regenerative medicine", "Laboratory of Tissues and Physiological Processes' Models" and "Cell and tissue engineering" - Master's and Bachelor's Degree Course in Biomedical Engineering.

She earned a Master Degree cum laude in Chemical Engineering (2001) and a PhD in Chemical and Materials Engineering (2006) from the University of Pisa, Italy. From 2006 to 2012, she has been postdoc fellow in the bioengineering research group managed by Prof. G. Ciardelli, initially at the University of Pisa and, then, since 2007 at Politecnico di Torino. From 2015 to 2018, she became Associate Professor at Politecnico di Torino and in 2018 she got the position of Full Professor as a recognition for being awarded the ERC Consolidator project BIORECAR.

During her academic career, she has been the coordinator of multiple research projects, among which STARIGEN FIRB2010 project (2012-2015), on the preparation of biomimetic scaffolds for cardiac regeneration and the ERC Consolidator project BIORECAR (772168; www.biorecar.polito.it; 2018-2024) on advanced strategies for myocardial regeneration. She has been recently granted ERC-PoC POLIRNA project, where she develops transfection kits for research use. Furthermore, in 2023 she has been granted 2 additional Proof-of-Concept grants and 1 PRIN 2022 project as coordinator, and 1 PRIN PNRR 2022 project as Unit Responsible, all focused on cardiac regenerative strategies through mini-invasive approaches.

Currently, she manages a research team including 1 Associate Professor, 2 Researcher2, 2 Postdoc Fellows and 6 PhD students. Furthermore she manages BIORECAR Cell Laboratory.

In 2021, she has been appointed Deputy Director of Centro 3R, the national Interuniversity Center for the Promotion of 3Rs Principles in Teaching and Research. Prof. Chiono has been involved in the organization of several conferences and symposia at national and international level.

Her research is highly interdisciplinary aimed at the design of innovative bioengineering approaches to solve key problems in regenerative medicine and nanomedicine, and includes the development of bioactive materials and interfaces, tissue engineering, materials characterization, in vitro tissue models, drug delivery and non-viral gene therapy. One main research topic is cardiac tissue regeneration through in situ miRNA release.

Prof. Chiono has supervised several undergraduate and graduate students (including multiple PhD students), postdoc fellows and researchers and delivered more than 40 oral presentations at international conferences, and authored >200 conference abstracts. She is author of 130 publications, including 83 articles and 34 abstracts in international peer-reviewed journals and 13 book chapters (H-index: 36 Scopus; 41 Google Scholar). She is also editor of 1 book on biofabrication. She filed 5 patents in the field of biomaterials, tissue engineering and nanomedicine for RNA therapy.

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tive procedures, requiring organic solvents, make them economically disadvantageous, strongly impacting on environment.
We propose a new class of nanoparticles able to incorporate and transport several types of molecules, starting on the same basic mixtures of phospholipids, triglycerides, fatty acids and cyclodextrins. Drugs or nutraceuticals can be added to these mixtures. Nanomicelles are obtained in water by spontaneous self-assembling of the mixture components, triggered by thermodynamic balance of dissolution-solubilization. These nanomicelles are characterized by a hydrophilic-co-hydrophobic core that is able to incorporate with high efficiency both hydrophilic and hydrophobic molecules. These nanomicelles have efficiently carried fenretinide, a derivative of Vitamin A, in tumour cells of neuroblastoma, lung cancer, colon cancer, melanoma and promyelocytic leukemia. Natural substances such as spermidine and berberine have also been delivered in neuroblastoma and osteosarcoma cells. The results of *in vitro* and *in vivo* studies indicated higher antitumor efficiency of the nanoencapsulated drugs than the free molecules. On the contrary, no toxicity was observed in normal cells. Finally, we have demonstrated optimal storage stability of these nanomicelles both at -20°C, and as a freeze-dried product to be rehydrated for use.

P14

Exploring the potential of aligned electroconductive nanofibrous membranes for culturing peripheral nervous system cells

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A large variety of substances (*i.e.* pollutants and therapeutics) have been reported to cause peripheral demyelinating neuropathies that result from the injury to Schwann cells and sensory/motor neurons, by direct toxic effect or immune-mediated cytotoxicity. *In vitro* myelination/demyelination assays are carried out using organotypic cultures of dorsal root ganglia rat explants, so far.
In this work, we proposed the development of an *in vitro* model reassembling the key features of the peripheral nerve composition by co-culturing Schwann cells (SC) and neurons on an engineered substrate having instructive cues to steer cell assembling. Instructive substrates were produced by electrospinning technology using a blend of Polycaprolactone (PCL) and Polyaniline (PANI) doped with Camphor-10-Sulfonic Acid (CSA). PCL, chosen as the core polymer of fibers, is characterized by high cytocompatibility and processability, whereas, PANI doped with CSA was added to enhance the conductive properties of the mats.
Random or aligned oriented fiber were produced and characterized in terms of physicochemical (hydrophilicity, stiffness, electroconductivity and composition) and structural (topography, alignment and diameter of the fibers).
Finally, preliminary *in vitro* experiments were carried out by seeding rat-derived SC onto our membranes to assess cell viability and proliferation by CellTiter-Blue® assay and cell organization by DAPI/Phalloidin staining.
Thanks to these preliminary tests, we can state that PCL-PANI electrospun membranes represent a promising system for culturing peripheral nervous system cells. In particular, the aligned mats helped the cells to have a more physiological morphology and organization, replicating the anisotropic structure of *in vivo* nervous tissue.
Funding: this project has received funding from Humatoxchip project - Bando Trapezio, Compagnia Sanpaolo and "Bando per il finanziamento ex-post di progetti di ricerca di Ateneo", Compagnia San Paolo.

P15

Electron microscopy studies of the bio-interactions of novel nanomaterials and *in vitro* models: the contribution of PMiB

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Electron Microscopy (EM) represents a powerful tool in biological studies to better understand how new Nanomaterials (NMs) are able to interact with *in vitro* models and studying their effects.
Transmission Electron Microscopy (TEM), with is high-resolution power (0.1 nm), allows to investigate bio-interactions between cells and NMs at the ultrastructure cellular level. Thanks to TEM analysis, it possible to describe how NMs affect the cells and if they are uptaken by cells entering the cellular membrane. Once inside the cell, NMs can be accumulated at cytoplasmic or nuclear level, causing possible structural damages bringing also to organelle malfunctions or even DNA damages.
Scanning Electron Microscopy (SEM), differently to the TEM, does not allow an investigation at the cellular ultrastructure level, but it can give information regarding the cell surface. With SEM analysis, it is possible to investigate damages at the cell membrane level and if NMs interact with cellular surface structures (*e.g.* filopodia) giving a different and complementary perspective of the bio-interactions from TEM studies.
Especially in nanotoxicology studies, knowing the structure and the composition of the NMs can be a relevant information. For these reason, Scanning Transmission Electron Microscopy (STEM) coupled with elemental analysis (*e.g.* Energy Dispersive x-rays (EDS) detector) can be used. It can univocally confirm the NM presence in the *in vitro* model both in TEM and SEM samples.
In addition, EM can be useful even to first characterize alone the NM to be tested. The novel NMs can be described in term of dimension, morphology, crystalline structure (diffraction pattern) and chemical composition (elemental analysis, *e.g.* EDS).
We report some cases of EM studies involving different *in vitro* models and several innovative nanomaterials.

P16

Evaluation of the biological effects of metal nanoparticles in an *in vitro* lung system at doses representative of environmental concentrations

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