Self-Assembling Peptides and Peptide Nucleic Acids: a versatile platform for 3D in vitro modeling and DNA/RNA detection.

Fabrizio Gelain[1,2]

[1]*Center for Nanomedicine and Tissue Engineering*

ASST GOM Niguarda, Milan.

[2]*Nanomedicine and Tissue Engineering Unit, ISBREMIT*

IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo

Self-assembling peptides (SAPs) are synthetic biomaterials (made of natural amino acids) at the forefront of tissue engineering because they are versatile, bioabsorbable, highly biocompatible, and biomimetic, i.e. amenable of functionalization with active moieties interacting with cells and proteins. SAPs provide nanostructured microenvironments morphologically resembling the natural ECM: they can be GMP-produced and tailor-made to match the requirements of the target cells (or tissues) of interest.

Human organoids from in vitro 3D cell cultures can generate a multitude of tissue and organ types, including brain regions and spinal cord tracts. Neural organoid systems have emerged as powerful models for drug discovery, toxicology, and the elucidation of specific disease mechanisms. For example, 3D cultures of patient-derived induced pluripotent stem cells (iPSCs) hold great promise for diseases modelling, drug screening, and, as such, opened new possibilities for personalized medicine. In our research center we developed the technology for culturing human neural organoids, encompassing human neural stem cells, multi-functionalized SAPs and dynamic cell cultures. By using synthetic scaffolds for organoid building, the pre-maturation of hybrid tissues to be used as patches in in vivo regenerative therapies is also achievable. Full characterization of neural organoids in long-term dynamic cultures is ongoing: it comprises histological mapping, microelectrode arrays (MEAs) recordings, calcium Imaging and gene expression analyses. Electrospun micro-guides composed of multi-functionalized SAPs, considered as mono-directional organoids, are also being tested for their neuroregenerative potential in vitro and in vivo.

On the other hand, the development of new efficient probes for DNA/RNA detection could have transformative implications for diagnosing complex pathologies. It may

enable early detection, reduce false positives/negatives, and facilitate tailored treatments. DNA/RNA probes can identify specific genetic mutations or biomarkers associated with complex pathologies, allowing for personalized treatment plans based on the individual's genetic profile. Additionally, pharmacogenomics can predict how patients will respond to drugs, optimizing therapeutic outcomes and reducing adverse effects. We are currently developing innovative nanobiosensors, designed to efficiently hybridize target DNA/RNA strands from the selected targets. We are integrating the potential of peptide nucleic acids (a synthetic analogue of DNA) with self-assembling peptide technology, together with cross-linking and electrospinning. Our goal is to develop and validate miniaturized biosensors to be integrated into microfluidic devices and POC to be used, for example, in environmental surveillance, gene isoform detection or precise diagnosis of genetic pathologies.