Biology goes to 3D

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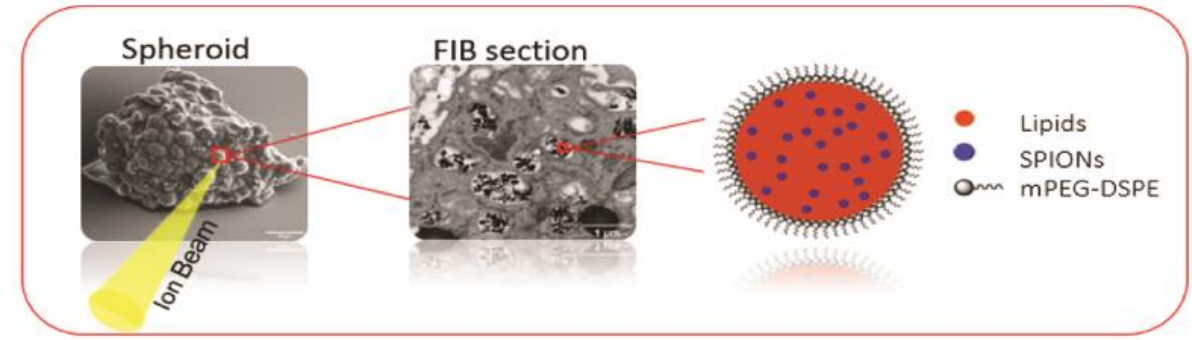

If someone asks you to reproduce a soccer ball what would you rather use: a sheet of paper or some playdough? Of course playdough, which make possible the reproduction of a 3D object.
Now, let's translate this idea to **biology**: what do you think would be better to reproduce a tissue, an organ or a tumor mass: a *two-dimensional (2D)* cell cultures or *3D cellular structures?* The answer it's easy: the 3D win the competition allowing to better reproduce the tridimensionality of *in vivo* systems. For this reason, spheroid-like cell architectures have become a powerful model system for biomimetic platforms towards the complete recapitulation of the organoid systems.
Spheroids are 3D cellular systems largely adopted as a model for high-throughput screening of molecules and diagnostics tools. Furthermore, those cellular platforms also represent a model for testing new delivery carries for selective targeting. Indeed, these closed systems are excellent tools for the understanding of complex cell functions and testing of new drug molecules.
The paper we are resuming here, focus on **3D tumor spheroids** and their interaction with nanomaterials: *lipid magnetic nanovectors* (LMNVs).

How to investigate the "dark side" of spheroids?

Optical microscopy techniques have been widely used to investigate spheroids but this technique shows several drawbacks in terms of characterization of the inner part of spheroid, resolution, and labeling of multiple cellular components. To overcome these limitations, the use of **electron microscopy techniques** seems to be very helpful allowing to mechanically "cut" thin slices of spheroids, yielding to hundreds of sections, which might be collected and analyzed individually and then virtually reassembled to reconstruct the entire structure. Nevertheless, this is an extremely time-consuming process.
In the paper we are reporting here, the authors "*mix and match"* cutting and image analysis using the most promising technique for 3D imaging: *FIB-SEM tomography*.
This technique combines the sectioning process, through a focus ion beam (FIB), and 3D analysis thank the *scanning electron microscope (SEM)* allowing to obtain information from the inner part of our spheroid up to the **subcellular level**.

The ROTO–UTP protocol

The crucial part of this entire process is the sample preparation, the authors proposed a **ROTO–UTP** *(reduced osmium–thiocarbohydrazide–osmium ultra-thin plasticization)* procedure for the specimen preparation which can be summarized in six cutting steps in relation to the use of different substances for fixation and heavy-metal staining. Simplifying even more, we can describe the whole process in three main phases: the fixation with different substances, the cutting process to have a rough reduction of the size of the spheroids and another fixation process in the same conditions.
The latest phase is the embedding in resin, and its polymerization to make the spheroids more resistant.
Thanks to this protocol several cellular structures have been revealed: defined **nuclei**, a large number of **mitochondria**, abundant rough **endoplasmic reticulum**, extracellular matrix and **tight junctions**. In addition to that appreciable vacuoles have been found, which would indicate a detectable eradication of **organelles** due to cutting process.

Spheroids and nanovectors (LMNVs) interaction

In the last part of the paper, the interface between LMNVs and the outer and inner domains of the tumoral spheroids has been investigated. Once again, thanks to the *FIB-SEM tomography*, the different phases occurred during the **endocytosis** (a cellular process in which substances are brought into the cell) of *LMNVs* have been recognized: the first contact between the nanovectors and the outer membrane, the **membrane deformation** toward the internal part (*invagination*) and then the formation of specific *clathrin-coated pits* (a protein that plays a major role in the formation of coated) and the final *LMNVs* internalization and their inclusion into vesicles.
This study proves fast **artifact-free processing** for the nanoscale investigation of 3D spheroids with nanomaterials which can be easily extended to organoids and micromaterials. This represents a powerful tool for tissue engineering, bioelectronic, and diagnostic platforms.

If you liked this article and you want deepen the topic, you can find the original work, on ***Advanced Materials Technologies***, here: https://onlinelibrary.wiley.com/doi/10.1002/admt.201900687