When and Where: Microfluidic Cancer-on-a-chip platform for real-time imaging of drug delivery systems stability and extravasation

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Drug delivery vehicles confront multiple physiological barriers after injection into the human body. Their performance strictly depends on the stability in that complex environment. Effective nanocarriers should be stable enough to successively overcome the encountered barriers avoiding premature drug release, while being smart enough to free the cargo once the target is reached. Ideally, the performance of a drug nanocarrier should be evaluated in an environment mimicking human physiology, to reduce and refine the number of preclinical and clinical trials. Commonly used 2D cell culture models do not reflect the dynamic and complex organization of a human body, meanwhile animal models are ethically arguable, expensive, time-consuming and still lack direct translation due to the differences between species. Those challenges in drug delivery screening, together with the emerging era of microfluidic technology are the driving forces for the creation of new solutions [1,2].

In our work we present a perfusable 3D cancer-on-a-chip platform, where we study time- and space-resolved stability of potential drug delivery nanocarriers. In the microfluidic chip we recreated a part of tumor microenvironment, where we focus on essential barriers challenging drug delivery systems stability (Figure 1). The microfluidic model recapitulates present *in vivo* perfusable blood vessel lined with organized Human Umbilical Vein Endothelial Cells (HUVECs), that create the cellular wall between the vessel lumen and the extracellular matrix, in which 3D HeLa cancer cells spheroids are distributed [3].

From the different drug delivery systems, we chose to evaluate supramolecular structures. We perfused the microfluidic platform with three amphiphilic PEG-dendron hybrids (previously studied in 2D cell culture), that change their fluorescent properties upon assembly into micelles [4]. Thanks to the compatibility of the microfluidic model with confocal spectral imaging we could register real-time stability and extravasation of the introduced nanostructures interacting with the 4 defined barriers (blood vessel, endothelial wall, extracellular matrix, tumor spheroid). This special property of the micelles allows us to follow their stability in correlation with the barrier they encountered, by real-time monitoring of changes in their fluorescence emission.

We observed a difference in extravasation of nanostructures depending on the leakiness of the endothelial barrier (studying healthy blood vessel and tumor blood vessel models), what correlates to the Enhanced Permeability and Retention (EPR) effect found *in vivo*. Interestingly, we could observe how the number and proximity of cancer cells affects the integrity of endothelial wall. Furthermore, we were able to identify most and least stable formulations by following their fluorescence emission. We registered decreased micelle internalization comparing to previously investigated 2D cell culture.

Our results demonstrate the applicability of the cancer-on-a-chip microfluidic platform in bridging the gap between 2D and *in vivo* studies. The vision of fast screening of a drug nanocarrier candidates in a 3D cell culture that could use patient derived cancer cells provides knew knowledge in the field of

nanomaterials' performance and brings us one step closer to the personalized nanomedicine.

References

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Figures

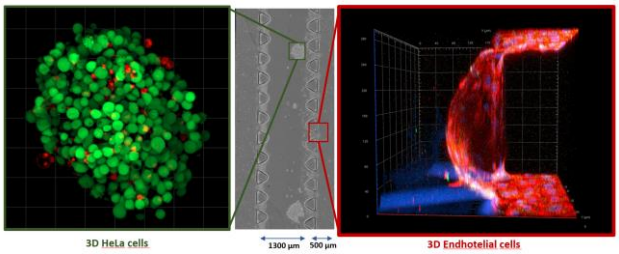

Figure 1. Cancer-on-a-chip platform. From left to right: A. HeLa cells spheroid confocal microscopy image (Green: live cells stained with Calcein, Red: dead cells stained with Propidium Iodide), spheroid diameter in vertical line: 240 µm. B. Transmission image of the cancer-on-a-chip platform. C. 3D reconstruction of HUVECs monolayer creating an interface between the extracellular matrix and the perfusable vessel lumen. Image acquired using confocal microscopy in Z-stack mode, in red: Phalloidin, blue: Hoechst, image height: 280 µm.