TUMOUR-ON-A-CHIP PLATFORM TO EVALUATE NANOPARTICLES PENETRATION IN 3D CO-CULTURE TUMOUR SPHEROIDS

Jean-Baptiste Blondé[1], Dwi Priwitaningrum[2], Jai Prakash[2], and Séverine Le Gac[1]

[1]*Applied Microfluidics for BioEngineering Research, University of Twente, The Netherlands*

[2]*Biomaterials Science and Technologies, University of Twente, The Netherlands*

ABSTRACT

Here, we report a breast tumour-on-a-chip platform to evaluate the penetration of nanomedicines in 3D tumour spheroids, as a function of the tumour spheroid cellular composition. Mouse multicellular tumour mono-culture spheroids and cancer cell-fibroblast co-culture spheroids were exposed to silica nanoparticles (NPs) (30 or 100nm) or liposomes (100nm) at various flowrates, mimicking the shear stress levels found *in vivo*. Results revealed that the NP penetration was influenced by the shear stress, and that the presence of fibroblasts in the tumour spheroids greatly reduced the penetration depth of the nanoparticles.

KEYWORDS: Microfluidics, Tumour Spheroid, Nanomedicine

INTRODUCTION

Nanomedicines have become a promising approach for drug delivery, providing safe transport of therapeutic agents safely through the body while allowing for active targeting toward the diseased areas [1]. Little is known however on the efficiency of the nanomedicine penetration in the targeted site, an essential information for the next generation of therapeutic agents designed to treat all cells deep in the tissue. In cancer research, 3D spheroids are more and more used as *in vitro* cellular models, as they phenotypically better mimic their *in vivo* counterparts, by containing key-features such as a proliferating shell and a hypoxic and necrotic core. Furthermore, they can be prepared to contain multiple cell types [2]. In previous work, we evaluated the penetration of nanoparticles (NPs) in tumour spheroids, under static conditions, in a microwell array. However, penetration was achieved through sedimentation on the tissues, and imaging was performed on cryosectioned tissues [3]. Here, in contrast, we propose a microfluidic approach allowing confining the spheroids in nL-volumes, perfusion of the nanoparticles and *in situ* imaging using confocal microscopy to evaluate the NPs penetration.

EXPERIMENTAL

Mouse breast tumour mono- and co-culture spheroids were prepared as described previously in a microwell array using cancer cells and fibroblasts (figure 1a) [3,4]. The spheroids were next injected in a PDMS microfluidic chip equipped with trapping structures (figure 1b), and suitable for confocal imaging.

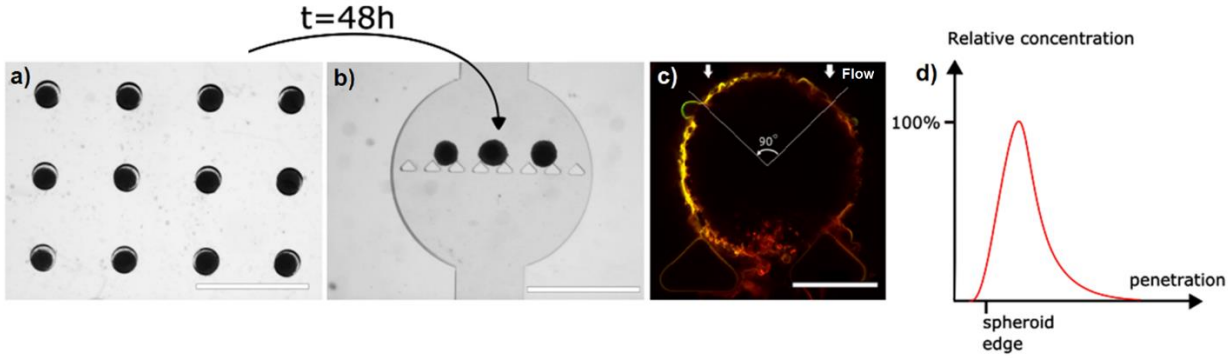

Figure 1 a) Mono-culture spheroids are prepared by seeding 4T1 mouse breast cancer cells in a microwell array platform [3]. Co-culture spheroids are prepared by combining 4T1 cells with 3T3 mouse fibroblasts in a 3T3:4T1 5:1 ratio [2]. b) After 48h incubation, 3 to 5 spheroids are injected in a microfluidic chip equipped with trapping structures, where they are exposed to a nanoparticle (NP) solution at different flowrates for 24h or 48h. c) After exposure, the penetration depth is quantified by averaging the NP penetration profiles on the side of the spheroid facing directly the flow and d) plotted as a function of the relative concentration of NPs per unit of depth.

Fluorescently labelled silica NPs (100-nm green & 30-nm red) were perfused in the microfluidic chip at different flowrates (0.1, 1 and 10µL/min) for 24 or 48h. Their penetration depth was evaluated using confocal microscopy and a home-written MATLAB script (figure 1d). In a second experiment, 100-nm rhodamine loaded DPPC/cholesterol liposomes were similarly perfused on the trapped spheroids.

RESULTS AND DISCUSSION

Low flowrate perfusion (0.1 µL/min) induced interstitial-like shear stress on the spheroids, and very little influence of the tissue composition was found on the NP penetration depth (figure 2a). By increasing the flowrate to reach a more vascular like shear stress (1 or 10 µL/min), penetration was reduced in presence of the fibroblasts, suggesting higher amount of extracellular matrix, as actually observed in the highest flowrate (10 µl/min, figure 2c). As expected, smaller NPs could penetrate deeper in the tissue, almost reaching the spheroid centre at the highest flowrate tested here. Next, we conducted another experiment using more relevant nanomedicine models; liposomes were similarly perfused, and deeper penetration was found for these "soft" NPs under the same flow conditions, compared to hard silica NPs, with the same influence of the tissue cellular composition (figure 2d).

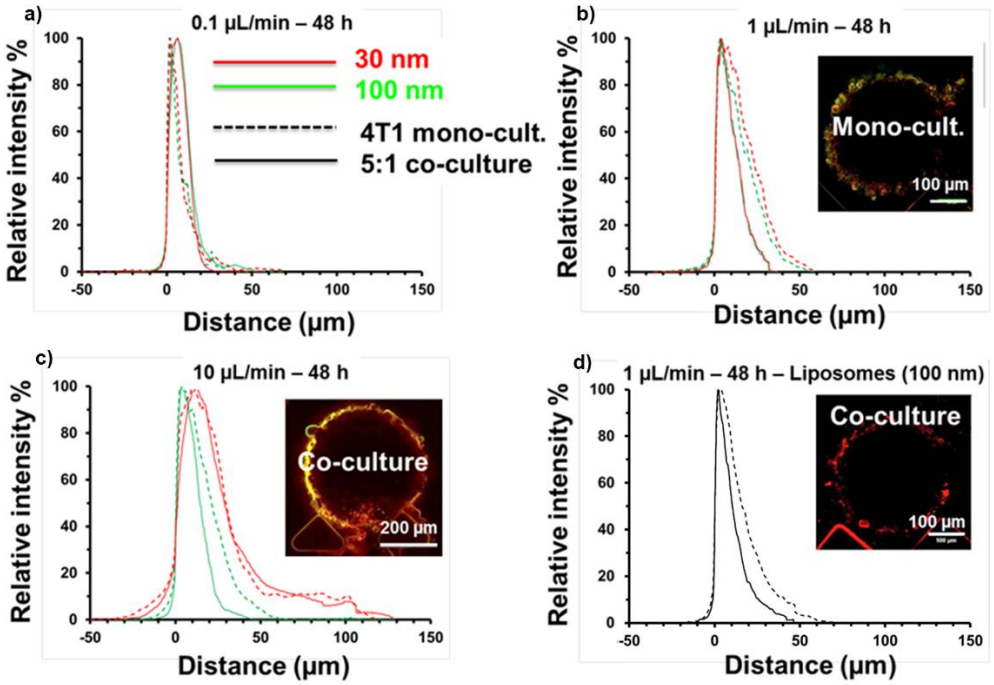

Figure 2 Nanoparticle penetration profiles after 48h continuous perfusion in the spheroid as a function of the cell composition, flowrate, and nanoparticle type: green (100nm) and red (30nm) fluorescent silica nanoparticles at a) 0.1µL/min, b) 1µL/min, c) 10µL/min, and d) rhodamine-loaded liposomes at 1µL/min. For all graphs, "0" on the x axis corresponds to the edge of the spheroids, and are the average of 6 spheroids.

CONCLUSION

In future experiments we will combine the tumour spheroid with blood vessels to better mimic the nanomedicine perfusion and the barrier induced by the blood vessel walls.

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