ENHANCING NANOMEDICINE PENETRATION IN TUMOR-ON-A-CHIP MODELS USING ULTRASOUND-MEDIATED MICROBUBBLE ACTIVATION

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ABSTRACT

Here, we report on ultrasound mediated microbubble activation for efficient and deep delivery of nanomedicines in co-culture multicellular tumor spheroids (MCTS), in a microfluidic chip. Specifically, a tumor-on-a-chip platform was realized by trapping multi-cellular tumor spheroids in a microfluidic chamber. Microbubbles and nanoparticles, used as models for nanomedicines, were perfused continuously, the microbubbles were activated by ultrasound and the penetration of the nanoparticles was quantified. Our experiments reveal that the nanoparticle penetration sites coincide with the initial positions of the microbubbles around the spheroids.

KEYWORDS: multi-cellular tumor spheroids, ultrasound, microbubbles, nanomedicines

INTRODUCTION

Nanometer-sized medicines hold great promises as imaging and therapeutic agents. However, their penetration in stroma-rich tumor is challenging, which limits their applicability. Next to this, the EPR effect (enhanced penetration retention), which has long been thought to improve nanomedicine delivery in tumors, is currently questioned [1]. Altogether, there is a need for new strategies for efficient and deep delivery of nanomedicines in stroma-rich tumor tissues. Activation of microbubbles in the vicinity of cells has been proven to locally induce cellular poration and promote intracellular drug delivery [2]. Here, we propose to use the same strategy to enhance the penetration of nanomedicines in tumor tissues. This study is conducted in a microfluidic chip, to produce a tumor-on-a-chip platform that resembles the confinement found *in vivo*.

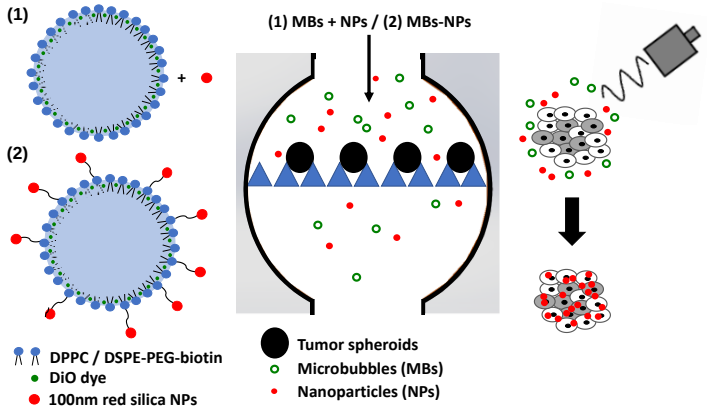

Figure 1: Schematic representation of the proposed work. Multicellular tumor spheroids (MCTS) are loaded in a microfluidic chamber (2000 micron diam.; 480 micron height), where they are exposed to a flow of microbubbles (MBs) and nanoparticles (NPs) (either (1) separated, or (2) conjugated to each other) for 20 min. Subsequently, MBs are activated by ultrasound on-chip to enhance NP penetration in the MCTS.

EXPERIMENTAL

Multi-cellular tumor spheroids (ca. 400-micron diam.) were prepared in a soft-embossed Petri dish [3] from a co-culture of mouse fibroblasts (3T3) and murine breast tumor cells (4T1) in a 5:1 ratio [4]. MCTS were trapped in a microchamber equipped with a row of pillars (Fig. 1); the device was fabricated from PDMS using soft-lithography and bonded to a RTS ThermoSealTM [5]. Microbubbles (1-2 micron diam.) were prepared using 85:15 mol% DPPC-DSPE-PEG-biotin; they were stained with DiO and filled with perfluorobutane gas. After MCTS trapping, a solution of microbubbles and fluorescent nanoparticles was perfused in the device for 20 min, before ultrasound activation (1 pulse of 2,000 cycles; 0-1000 kPa; 1-2 MHz). Nanoparticle penetration was assessed *in situ* using confocal microscopy. To that end, MCTS were counterstained with NucBlue™ to define the edge of the MCTS and evaluate light penetration in the 3D tissues. Data were analyzed using Matlab® to quantify the nanoparticle penetration depth and characterize the spatial delivery of nanoparticles, as depicted on Figure 2.

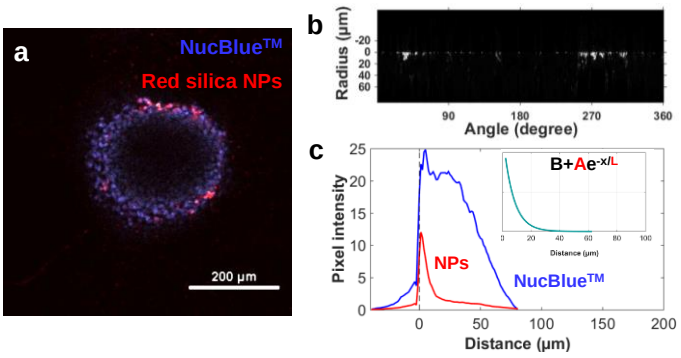

Figure 2: Data analysis. Data are analyzed using a Matlab script: from the confocal microscopy picture (a), NP penetration is visualized in a polar coordinate representation (b). Next, a graph is plotted after integration of the fluorescent signal around the entire spheroid (c). An exponential fit (inset in c) allows for determining the NP penetration (L) and the maximum intensity of NP penetration (A).

RESULTS AND DISCUSSION

In a first series of experiments, 100-nm sized red fluorescent silica nanoparticles (carboxy-terminated) used as nanomedicine models were perfused jointly with the microbubbles. Nanoparticles were found to locally accumulate inside the MCTS. These sites of accumulation corresponded to the initial positions of the microbubbles, as evidenced by the lipids detected at the same locations in the MCTS (Fig. 3). While after 48 h static incubation, nanoparticles accumulated at the periphery of the MCTS and penetrated by diffusion in it [5], the use of microbubbles and ultrasound readily enabled deep penetration of the nanoparticles in the MCTS. In a second series of experiments, similar silica nanoparticles were attached to the microbubbles using biotin-streptavidin coupling to spatially control the nanomedicine delivery in the tumor tissue, while maximizing the delivery efficiency. As before, the nanoparticle accumulation sites coincided with the microbubble initial positions (Fig. 4).

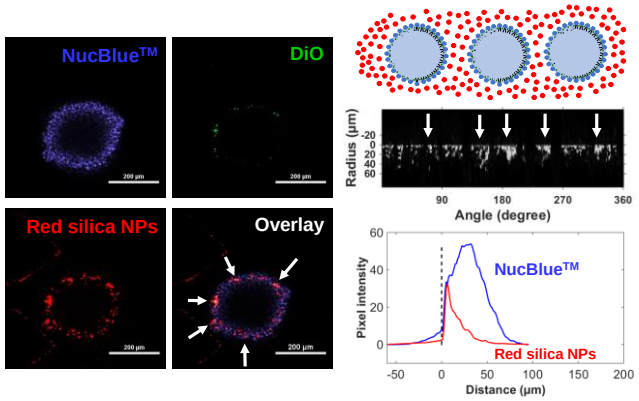

Figure 3: Experiment 1: nanoparticles and microbubbles, not conjugated. Penetration of Si NPs (100 nm; carboxy-terminated) using MBs in MCTS. Ultrasound parameters: 500 kPa; 2 MHz. Picture acquired at 1/6 from the top of the spheroid. White arrows point to the NP accumulation sites in the MCTS.
Here, A = 22.86 ± 11.31 (a.u.; pixel intensity); L = 11.58 ± 4.47 µm.

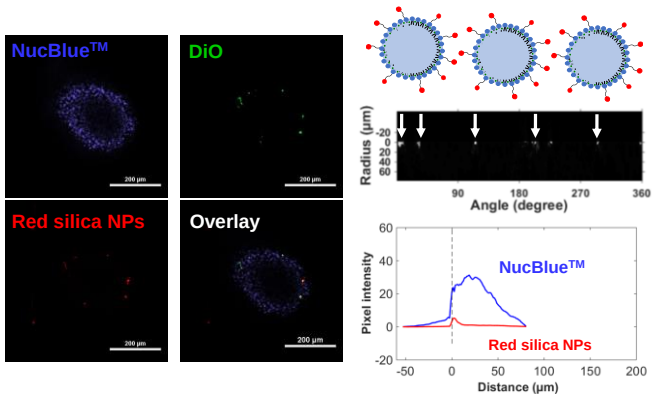

Figure 4: Experiment 2: nanoparticles conjugated to microbubbles. Penetration of Si NPs (100 nm; carboxy-terminated) using NP-conjugated MBs in MCTS. Ultrasound parameters: 1000 kPa; 1 MHz. Picture acquired at 1/6 from the top of the spheroid. White arrows point to the NP accumulation sites in the MCTS.
Here, A = 4.68 ± 2.76 (a.u.; pixel intensity); L = 12.43 ± 11.51 µm

CONCLUSION

We are currently studying the impact of the exact ultrasound parameters (frequency & applied pressure) on the nanomedicine penetration depth. Next, we plan to apply the same strategy for the delivery of drug-loaded liposomes.

REFERENCES

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