CHARACTERIZATION OF NANO-PHOTOSENSITIZER DELIVERY AND PHOTODYNAMIC EFFICACY USING MULTICELLULAR TUMOR SPHEROIDS (MCTS)

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ABSTRACT

We present a new method of evaluating delivery and efficacy of nanometer-size photosensitizers on multicellular tumor spheroids (MCTS) utilizing microfluidic technology. A custom designed microfluidic device with different size microchambers is adopted in our tests. Different sizes of SKOV3 spheroids are formed simultaneously inside the chip using an optimized cell loading and culturing protocol. Surface-engineered nanoparticles are delivered into the chip and their photodynamic efficacy performance on various spheroids are evaluated by single screening experiment. Using this method, we successfully demonstrated the varying photodynamic efficacy for different formats of photosensitizer (free dye and nanoparticles), spheroid sizes, and 2D/3D tumor models.

KEYWORDS: PDT, nanoparticle, drug efficacy, microfluidic, cancer, MCTS

INTRODUCTION

Three-dimensional (3D) tumor models, such as multicellular spheroids, have received an increasing attention in drug development since they could better reflect in-vivo tumor microenvironments and potential drug efficacy than conventional 2D monolayer cell culture models [1]. Microfluidic technologies have been brought into this application and make it more feasible to provide precisely-controlled spheroid sizes, long-term culturing with dynamic perfusion, and in-situ drug tests in 3D culture [2, 3]. Also their advantages in high-throughput screening and precise microenvironment controls can be readily applicable for both free dye and nanoparticular photosensitizer assays [4-6]. Here we report our recent study on nano-photosensitizer delivery/efficiency characterization using multicellular tumor spheroids (MCTS). Two main features of our microfluidic platform used for this study are: (1) simultaneous formation of MCTS in different sizes for high-throughput; and (2) comparative photodynamic efficacy assays between 2D and 3D tumor models in parallel.

DEVICE DESIGN

Figure 1(a) shows a prototype device with an array of 64 microwells which allows for spheroid formation, culture, and drug test. Along the channel, the diameter of microwells gradually increases from 160 to 440 μm, allowing formation of different-size spheroids.

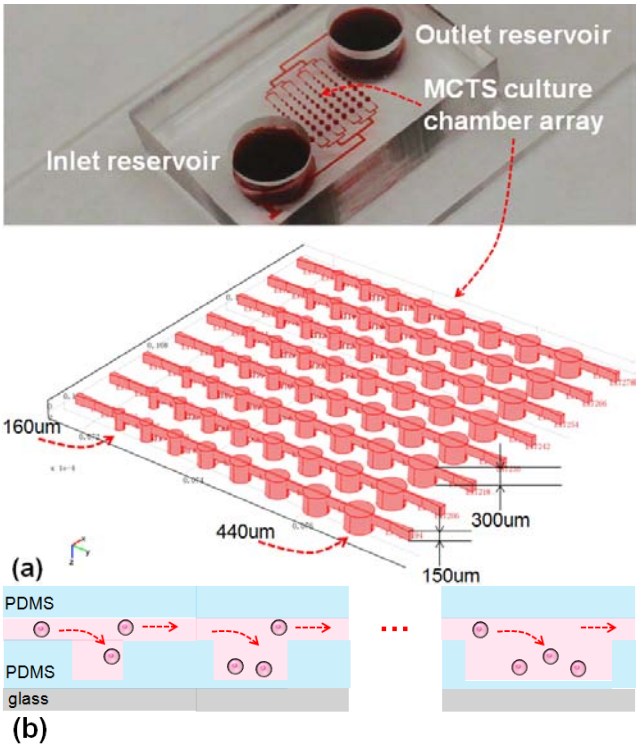

Figure 1. (a) Image of a prototype device and a schematic view of the proposed MCTS culture chamber array chip, (b) Cross-sectional view of cell loading process.

As shown in Figure 1(b), during the cell loading process some of the cells will settle down in the microwells while the rest will flow over. A larger size microwell allows for both a larger number of starting cells and a larger space for spheroid formation later. The whole chip is composed of two PDMS layers bonded face-to-face, and the surfaces of inner structures are pre-treated with 1% (w/v) Pluronic F108 solution, which prevents cell attachment and allows for spheroid formation. A similar microfluidic chip was used in 2D cell culture for comparative tests. The major difference is that one PDMS layer is replaced with a glass substrate and the surface is pretreated with 100 µg/ml Collagen type I solution for cell attachment.

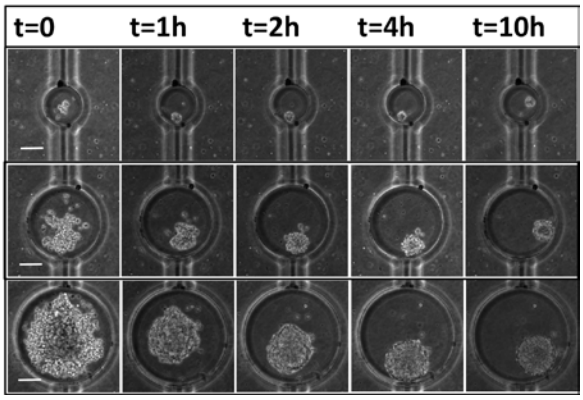

Figure 2. Time lapse recording of SKOV3 spheroid formation in different size microwells. Scale bar: 100um.

EXPERIMENTAL AND RESULTS

SKOV3 ovarian carcinoma cells are prepared in suspension with a concentration of 5×10^6/ml for cell loading. The cell loading protocol is composed of two steps. In step one, the inlet is filled with 150 µl cell suspension media, while and the outlet is filled with 50 µl culture media. This results in a feeding flow of under 35 Pa pressure difference, driving from smaller microwells to larger microwells. This step continues for 5 minutes. In step two, the inlet is filled with 50 µl culture media and the outlet is filled with 150 µl cell suspension media, which drives a reversed feeding flow from larger microwells to smaller microwells. This step continues for another 8 minutes. After that, both inlet and outlet are filled with fresh culture medium (150 µl and 50 µl, respectively), which helps remove any residual cells in the microfluidic channels and continuously provides media supply in the following over-night cell culturing and spheroids formation. As shown in Figure 2, cells in the same microwell gradually aggregate together and form a single compact spheroid within 10 hours. Based on the different starting cell numbers, various spheroids in sizes from 60 to 300 µm can be formed simultaneously in the same chip.

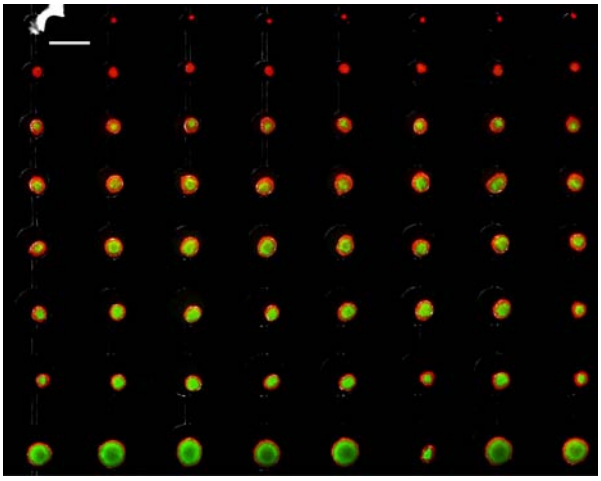

Figure 3. SKOV3 cell viability distribution in different size spheroids after PDT treatment. Scale bar: 400um.

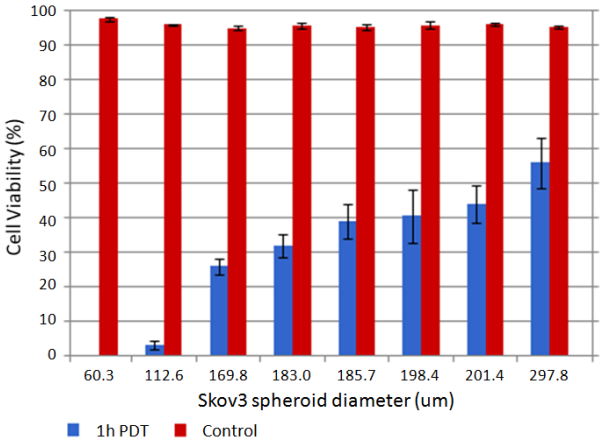

Figure 4. Cell viability as a function of different MCTS sizes, comparing the assay result of 1 hour PDT treatment and control group (no PDT illumination).

Distinctive differences in cell viability were observed for different sizes of SKOV3 spheroids after the same PDT treatment. 5 mg/ml nano-photosensitizer solution was prepared in the same way as reported in our previous work [5] and loaded into the device. After 1 hour incubation time, residual nano-photosensitizers were washed away with fresh RPMI for 10 minutes. Then, all the spheroids in the device were exposed to the same illumination (wavelength 637 nm) for 1 hour (total 118 J/cm2) for activation of

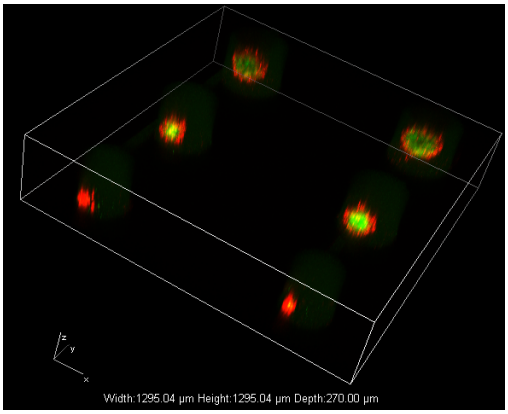

Figure 5. Confocal microscope image of cell viability distribution within SKOV3 spheroids after PDT treatment.

photosensitizers. We stained the cells with Calcein AM (live/green) and Ethidium homodimer-1 (dead/red) for cell viability evaluation. Figure 3 shows the results. Larger size spheroids appear to be more drug resistant to the same PDT treatment than smaller size spheroids. At the same time, all spheroids in the control group (exposed to the same nano-photosensitizer incubation but without PDT illumination) retain a high viability, as shown in Figure 4. The cell viability distribution of different spheroid sizes is further confirmed under a confocal microscope (Nikon A1R-A1). As shown in Figure 5, the smallest spheroids appear to be all red (i.e., dead), while the larger ones have some viable cells (green) in the core, surrounded by dead cells (red spots). Compared to a 2D monolayer model, 3D spheroids demonstrated overall higher PDT resistance to both nano-photosensitizers and small molecule photosensitizer (methylene blue), as shown in Figure 6. Also, it is observed that nano-photosensitizers are more effective in peripheral parts of spheroids, compared to the small molecule photosensitizer.

CONCLUSION

We implemented a microfluidic technology to investigate nano-photosensitizer delivery and photodynamic efficacy on 3D MCTS models in a high-throughput way. Custom-designed chips are prepared to successfully create MCTSs of different sizes simultaneously. Distinctive photodynamic efficacy has been observed for different formats of photosensitizer (free dye and nanoparticles), spheroid sizes, and 2D/3D tumor models. This method can be easily applied for guiding nanodrug development and clinical therapy optimization.

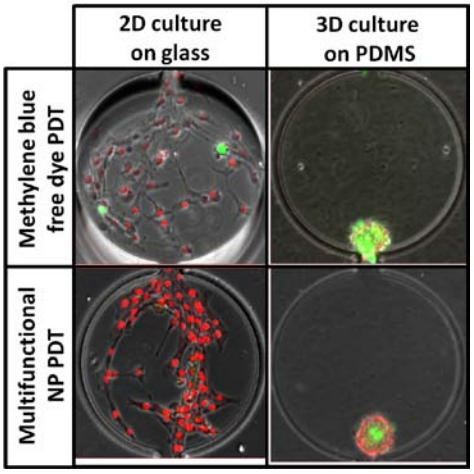

Figure 6. Cell viability results after PDT treatment, comparing the efficiency under two in-vitro models (2D monolayer and 3D spheroid models) and two types of photosensitizer (Methylene Blue free dye and Methylene Blue loaded nanoparticle photosensitizers), respectively

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