INVESTIGATING PHOTODYNAMIC EFFICIENCY OF TUMOR TARGETED NANOPARTICULAR PHOTOSENSITIZER USING MICROFLUIDIC CHIPS

X. Lou[1], G. Kim[2], Y. Koo Lee[2], R. Kopelman[2], and Euisik Yoon[1*]

[1]Center for Wireless Integrated Microsystems, Dept. of EECS, University of Michigan, Ann Arbor, MI, USA

[2]Dept. of Chemistry, University of Michigan, Ann Arbor, MI, USA

ABSTRACT

We present a new high-throughput assay for screening photodynamic efficiency of nanodrugs in a microfluidic chip. Surface engineered nanoparticles are applied to multiple cell lines for evaluating their specificity on target cancer cells. Using a microfluidic gradient generation network in the fabricated prototype chip, photodynamic efficiency for various nanodrug doses was simultaneously evaluated in one single screening. Temporal control of drug injection into the microfluidic channels provides a precise control of drug treatment time on target cancer cells, which is one of critical parameters in PDT treatment. Using the proposed microfluidic assays, we successfully demonstrated the nanodrug's photodynamic efficacy testing for different target cell types, drug doses and treatment protocols.

KEYWORDS: PDT, photodynamic, drug efficacy, microfluidic, nanoparticle, cancer, drug screening

INTRODUCTION

Photodynamic therapy (PDT) is rapidly expanding its applications from dermatological cancer treatment to other cancers including brain, prostate, cervix, etc[1]. Besides various clinical trials, development of next-generation photosensitizers (PS), including targeted nano-fabrication of PS [2], has been major research focus [3]. Microfluidic technologies have been brought into this application in order to benefit from low cost, high throughput and well-controlled microenvironments [4-5]. Although some of the previous work demonstrated powerful screening capability for general PDTs, more customized features are required for nanodrugs according to its cytotoxicity mechanism. Here we report our recent work on photodynamic assays for tumor-targeted nanoparticular (NP) photosensitizers. Two main features of our approach include: (1) simultaneous screening of multiple cell types for targeted delivery of functionalized NPs and (2) temporal control of drug feeding for evaluating NP drug delivery to cancer cells.

DEVICE DESIGN

Figure 1 shows the proposed multi-layer PDMS chip composed of three layers: a fluidic layer (at the center for cell culture and PS concentration control), a gas layer (at the top for providing oxygen level control), and a filter layer (at the bottom for providing exposure dose control). Structure and detailed fabrication processes of the prototype chip were reported in [5]. In this work, we modified the fluidic layer according to the need for screening multiple cell lines in different nanodrugs. We implemented parallel culture channels which are isolated from each other and having individual inlets and outlets to load different cell lines in the same chip. We also adjusted the channel length and flow resistance in order to provide different drug incubation time for the loaded cells, from 0 to over 75mins, depending on their position in the culture channel.

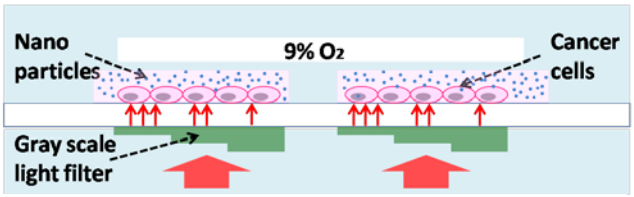

Figure 1. Cross-section view of a typical microfluidic chip we used in this work for PDT assays.

NANOPARTICLE PREPARATION

Methylene blue (MB)-conjugated polyacrylamide (PAA) nanoparticles were prepared by a reverse microemulsion polymerization method. A monomer solution containing acrylamide (610 mg), co-monomer N-(3-aminopropyl) methacrylamide hydrochloride (APMA) (45 mg), MB succinimidyl ester (MB-SE) solution (60µL, 50 mg/mL in DMSO), and Fluorescein isothiocyanate (FITC) solution (10 µL, 100 mg/mL in DMSO) were dissolved in a phosphate buffered saline solution (1.4 mL) and stirred for 2 hr at 37°C. Crosslinker 3-(acryloyloxy)-2-hydroxypropylmethacrylate (AHM, 225mL) was added into the mixture later. The dye labeled monomer solution was added to a deoxygenated hexane (36 mL) with surfactants, sodium dioctyl sulfosuccinate (AOT, 1.3 g) and Brij 30 (3.2 mL). Then, radical initiator ammoniumpersulfate (APS, 10% w/v, 100 mL) and N,N,N',N'-tetramethylethylenediamine (TEMED, 100mL) were added to the mixture solution to trigger radical polymerization and run for 2 hrs. Produced NPs were purified by several washing procedures and recovered in powder.

The surface of prepared NPs were further modified for targeted binding for specific cancer cells. 50 mg NPs were dissolved with 4 mg bifunctional PEG crosslinker (SCM-PEG-MAL) in 2.5 mL PBS (pH 7.4). After 30 min reaction with stirring, PEG conjugated NPs were purified by centrifugal filtration (100 kDa) for removing unconjugated bifunction PEG. Then,

11 mg of F3 peptide (cancer targeting moiety) was added into the NP solution and the conjugation reaction was run for an overnight and the reaction mixture was treated with L-cystein (1.74 mg) for additional 2 hrs. The final product was recovered after washing five times by centrifugal filtration (100 kDa) and kept frozen until use. Figure 2(a) shows schematic of PAA NPs covalently incorporating a photosensitizer (Methylene Blue (MB)) and fluorescent dye (FITC) inside, and coated with F3 peptides for specific targeting tumor cells. NPs have an average diameter of 20-30nm (Figure 2(b)).

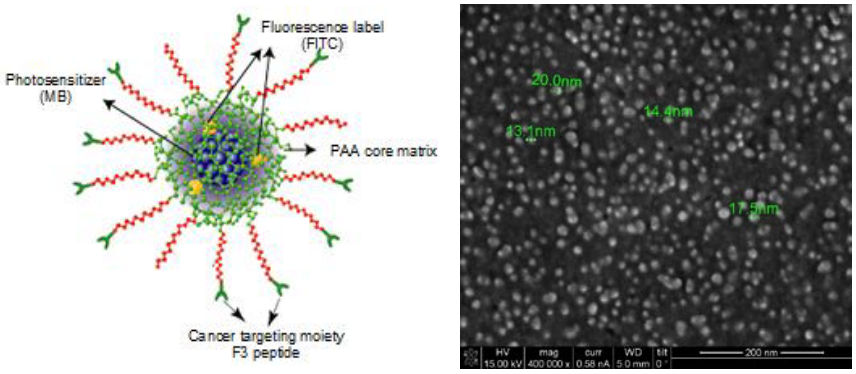

Figure 2. (a) Schematic of the functionalized nanoparticles. (b)SEM images of the MB-loaded fluorescent nanoparticles.

EXPERIMENTAL AND RESULTS

Rat C6 glioma tumor cells and DI TNC1 cells were used for targeted NP delivery test. Both cells were introduced into the chip with a concentration of ~$2.0x10^6$ cells/ml, and cultured in culture media (DMEM+5%FBS+0.5%penicillin) for 24h to form a monolayer. After that, 1mg/ml NP in DMEM solution was introduced into the chip, cultured with both cells for 1h and washed away. For PDT activation, cells were exposed to an uniform illumination from a red LED array (with a peak output at 660nm) for 30 mins (total dose of 42.8mJ/cm^2). Then, cells were cultured for 20mins, and stained with Calcein AM (10uM, live, green) and Ethidium homodimer-1 (10uM, dead, red) for PDT efficiency evaluation. Figure 3(a) shows the adjacent culture channels loaded with DI TNC and C6 cells, respectively, and filled with the same concentration of FITC fluorescence NP solution (pseudo-colored blue displaying similar fluorescence intensity). NPs were selectively attached on C6 cells. This can be verified by blue fluorescence observed only for C6 cells in the bottom channel after washing the channels (Figure 3(b)). As a result, C6 cells can be "selectively" killed during the following PDT treatment, while DI TNC cells can be unharmed. Figure 3(c) shows the cell viability results and confirms the drug specificity.

Photodynamic efficiency under different NP concentrations were also investigated for C6 cells as a target cancer. C6 cells were loaded into the chip and the monolayer was formed in the same way as described previously. Two different culture media (DMEM and DMEM mixed with 1mg/ml NPs) were introduced to the fluidic layer using a gravity flow generated from the liquid height difference between inlets and outlets. An optimized pressure difference of ~200Pa was applied to guarantee the complete mixing in the microfluidic gradient generation network. After 1h's incubation with NP solutions, both inlets were refilled with fresh DMEM, which washed away any unbounded NPs in the culture channels in the same way they were introduced. This allows the same incubation time for NPs for all the cells in the channel. After that, cells were exposed under the same LED array light source for 30mins, continuously cultured for 20min, and evaluated for viability using the live/dead staining. Statistical analysis of cell viability were performed by counting the ratio of live (green) cells to the total number of cells using ImageJ software. Figure 4(a) shows the gravity flow setup with the elevated inlet reservoirs. FITC fluorescence image was captured in the serpentine channel area where two different NP concentration solutions were mixed. As shown in Figure 4(b), fluorescence intensity gradually become homogeneous along the channel, indicating complete mixing of two concentrations. Figure 4(c) shows the variation of fluorescence intensity in the culture channels. Different NP concentrations can be loaded for incubation (left), and the corresponding image after unbounded NPs are washed away with fresh media (right). Figure 4(d)

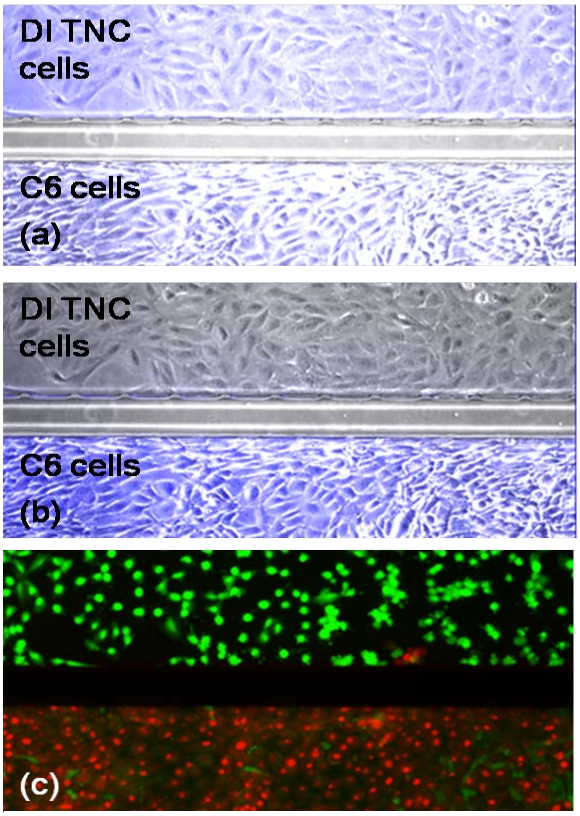

Figure3. (a) DI TNC1 cells (upper) and C6 cells (bottom) after incubated with the same NPs (fluorescence pseudo-colored with blue) solution, (b) Cell images after NP solution is washed away, and (c) Cell viability results after 42.8mJ/cm^2 exposure treatment (Green: live cells, red: dead cells).

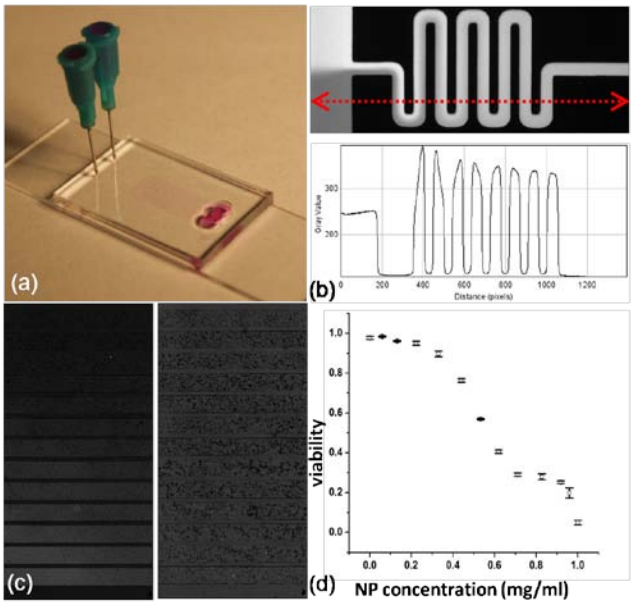

Figure4.(a) Gravity flow setup, (b) Fluorescent image of NP mixing in the chip, (c) NP incubation with a gradient (left) and after being washed away (right), (d) C6 cell viability as a function of different NP concentration.

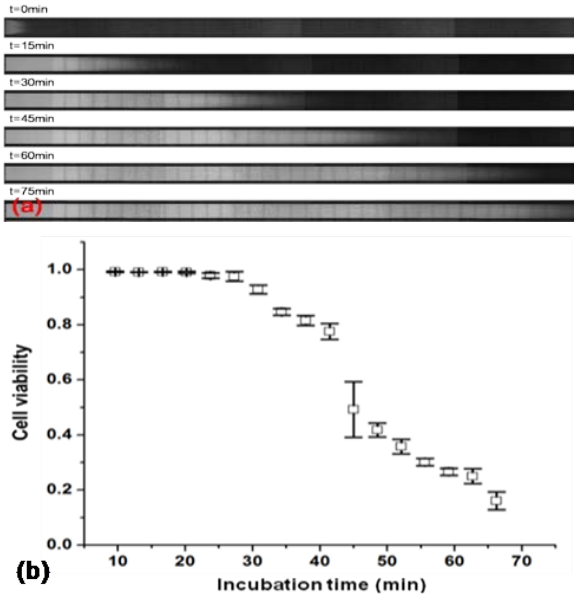

Figure5.(a) Time lapse images of NP solutions flowing through the cell incubation channels, (b) Cell viability changes according to different incubation time along the channel.

shows the cell viability as a function of NP concentration, ranging from 0mg/ml to 1mg/ml, showing a gradual viability drop as the NP concentration increases.

We tested PDT efficiency under different durations of incubation time. If the NP solution slowly injected through the channel by a gravity flow, the cells in upstream and downstream would have experienced a different incubation time. As shown in Figure 5(a), NP solution is gradually covering the whole culture channel within 75 mins. After 75 mins, the whole culture channel was refreshed with pure DMEM within 1min by a pressure-driven flow. Figure 5(b) shows that cell viability drops gradually as the incubation time increases. This implies a minimum incubation time is required to ensure all the NPs be attached to target cancer cells and acquire a sufficient molar ratio for effective PDT treatment.

CONCLUSION

We implemented a microfluidic technology to investigate photodynamic efficiency of specific tumor-targeted nanoparticular photosensitizers. Custom-designed chips successfully screened NP related factors affecting PDT efficiency including selective targeting to specific cancer cells, optimal incubation time for NP attachment, and effects of NP concentration on cell viability. The prototype devices can be applied for high-throughput nanodrug development and clinical therapy optimization.

ACKNOWLEDGEMENTS

This work has been supported in part by Thermo Fisher Scientific Screening Technology Grant and Academic Excellence Alliance Award from KAUST. The authors would like to thank Yipei Wang on kindly providing DI TNC1 cell line, and Dr. Ji Yoon Kang and Dr. Maesoon Im for their helpful discussion.

REFERENCES

[1] A. Chiaviello, et al, "Targets and mechanisms of photodynamic therapy in lung cancer cells: a brief overview," Cancers, **3**, 1014(2011)

[2] H. Hah, et al, "Methylene Blue-Conjugated HydrogelNanoparticles and Tumor-Cell TargetedPhotodynamic Therapy," Macromolecular Bioscience, **11**, 90 (2010).

[3] D. Dolmans, D.Fukumura, R. Jain, "Photodynamic therapy for cancer," Nature Reviews Cancer, **3**, 380 (2003).

[4] R. Jarvis, X. Niu, A. Mello, M. Wainwright, R. Wootton, "A microfluidic flow system for activity screening of Photo-Dynamic Therapy agents," Proceedings of uTAS'09, 603 (2009).

[5] X. Lou, G. Kim, Y. Koo, R. Kopelman, E. Yoon, "High-throughput screening of photodynamic therapy (PDT) screening from multiple parameter assays of 1,000 different conditions in a single chip," Proceedings of uTAS'10,719, (2010)

CONTACT: E. Yoon, tel: +1-734-615-4469; esyoon@umich.edu