DEVELOPING A MICROFLUIDIC-BASED PLATFORM FOR HIGH THROUGHPUT SCREENING OF NANOPARTICLE TOXICITY

Ziqiu Tong[1], Gayathri Rajeev[2], Keying Guo[1], Angela Ivask[2,#], Scott McCormick[2], Enzo Lombi[2], Craig Priest[2], and Nicolas H. Voelcker*[1,2]

[1] The Monash Institute of Pharmaceutical Sciences, Monash University, VIC, Australia
[2] Future Industry Institute, University of South Australia, Mawson Lakes, SA, Australia
[#] Current address: Laboratory of Environmental Toxicology, National Institute of Chemical Physics and Biophysics, Tallinn, Estonia

* Email: nicolas.voelcker@monash.edu; Tel.: +61 3 99039230

Manufactured nanoparticles (MNPs) are microscopic materials (sub-100 nm) having physical and chemical properties uniquely different from their respective bulk materials. MNPs have found applications in optical, electronic, and biomedical fields and numerous novel products containing MNPs are entering the market each year [1, 2]. However, there are many reports of cyto- or genotoxicity of MNPs [3]. A rapid and reproducible screening tool for analyzing and determining MNP toxicity *in vitro* in a high throughput manner would be desirable to safeguard against toxic effects to humans.

This paper reports a novel approach of using a simple design of microfluidic device to assess the cyto- and genotoxicity of MNPs in a high throughput manner. The microfluidic toxicological testing platform is based on mammalian cells that are immobilized inside microchannels and subsequently used for toxicological analysis of MNPs. Our group has previously developed a lab-on-a-chip toxicological testing platform that contains different cell types immobilized to the surface using antibody-cell surface antigen recognition [4]. While this lab-on-a-chip platform and other commonly used well-plate culture techniques [5] used for MNP toxicological testing are performed under static conditions, there are reports showing that flow through systems may be significantly more relevant highlighting the advantages of using a microfluidic platform approach [6].

A schematic diagram of the crossed flow design of the microfluidic system is shown in Figure 1 where a glass substrate is functionalized with cell adhesion molecules to bind to various types of cells, and parallel streams of the cell suspensions and nanoparticle suspensions are perfused into the chip sequentially. As a proof of concept, a polydimethylsiloxane (PDMS)-based microfluidic chip containing 5 inlets for cell suspension and 5 inlets for nanoparticle solutions is designed, fabricated, and characterized (Fig. 2).

Collagen 1 or antibodies against cell surface antigens were first immobilized on epoxy-silane functionalized glass substrates using a microarrayer system prior to the perfusion of cells (Fig. 3). An array of 25 spots (diameters of 350 μm) of cell clusters were thus generated. Using two cell dyes as model treatments, we have demonstrated to be able to deliver up to 5 conditions per treatment simultaneously to the immobilized cells array (Fig. 4). Furthermore, we have used this platform to assess cell viability when subjected to simultaneous treatments with solutions of different tonicity (hence toxicity). Finally, we have shown to perfuse solutions of microparticles with various surface modifications (decorated with different antibody concentrations) to the cell microarray (Fig. 5). We have chosen microparticles as proof of concept working towards using nanoparticles in the future.

We are now scaling up the number of microchannels for immobilization of larger number of cell populations and for screening of large number of nanoparticles. This cross flow microfluidic configuration platform can provide a faster readout, is cost effective, offers time-resolved monitoring, and retains high magnification imaging capability. It could potentially lead to the reduction of animal model experiments and achieve a new testing standard for nanoparticle toxicity.

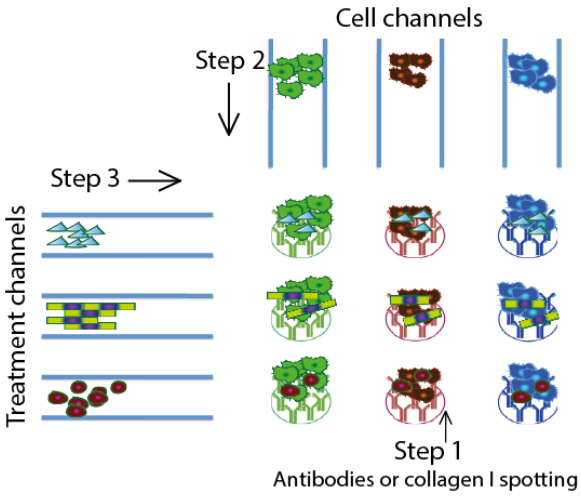

Fig. 1 Schematic drawing of crossed flow concept of the microfluidic testing platform where flowing cells were captured by spotted collagen or antibodies and assessing their interactions later with nanoparticles under flow.

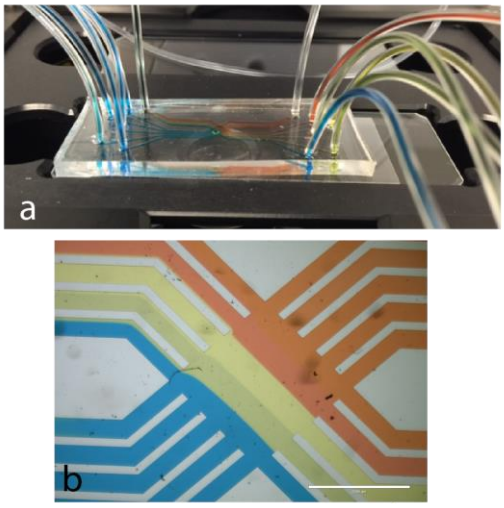

Fig. 2 Photograph of the microfluidic device integrated with the inlets and the outlets (a). Food coloring dyes were used for testing the laminar flow profile of 5 parallel fluid streams (b). Scale bar: 2 mm.

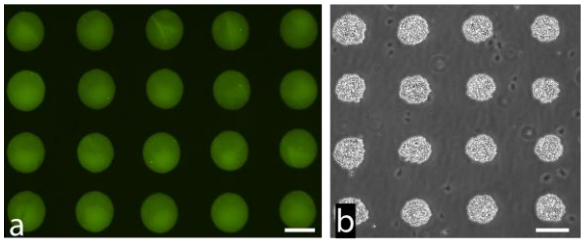

Fig. 3 Cell adhesion molecules (e.g., collagen 1 or antibodies) were printed onto glass substrate via a microarray spotter (a) and were used for subsequent capturing of cells inside a microfluidic chip (b). Scale bars: 350 µm.

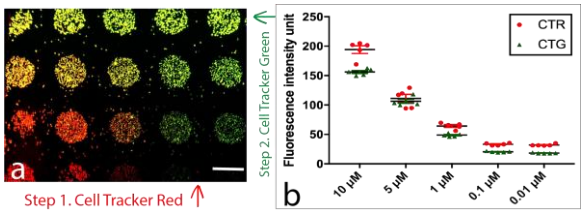

Fig. 4 The cell microarray was first treated with different concentrations of Cell Tracker Red and then with different concentrations of Cell Tracker Green orthogonally (a). Fluorescence intensity per cell spot was calculated and compared (b). Scale bar: 350 µm.

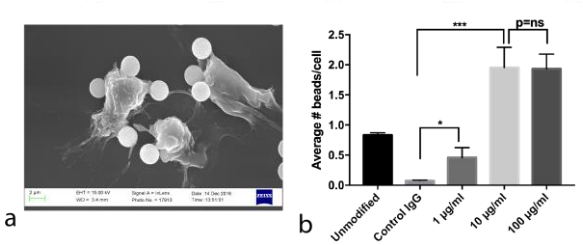

Fig. 5 Solutions containing microbeads with different surface modifications were used to perfuse over the cell microarray using the microfluidic system.

REFERENCES:

[1] Project on Emerging Nanotechnologies. Consumer Products Inventory. October 2013, from http://www.nanotechproject.org/cpi

[2] Malysheva, A. et al. Bridging the divide between human and environmental nanotoxicity. Nature nanotechnology. 2014, 10, 835-844

[3] Levard, C. et al. Environmental Transformations of Silver Nanoparticles: Impact on Stability and Toxicity. Environ Sci Tech. 2012, 46, 6900-6914.

[4] Vecchio, G. et al. Lab-on-a-Chip-Based High-Throughput Screening of the Genotoxicity of Engineered nanomaterials. Small. 2014, 13, 2721-2734

[5] Burke, T. et al. Development and Application of Fluorescence Polarization Assays in Drug Discovery. Combinatorial Chemistry & High Throughput Screening. 2003, 6, 183-194.

[6] Mahto, S. et al. A new perspective on in vitro assessment method for evaluating quantum dot toxicity by using microfluidics technology. Biomicrofluidics 2010, 4, 034111