REAL-TIME MULTIMODAL IMAGING OF NANOPARTICLE-CELL INTERACTIONS IN HIGH-THROUGHPUT MICROFLUIDICS

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ABSTRACT

We present an interdisciplinary approach that allows for the evaluation of nanoparticle cytotoxicity at the single-cell level. By combining single-cell microfluidics with nanoparticle chemistry, real-time and high-throughput multimodal imaging of cellular interactions with functionalised gold nanorods was successfully achieved. The integration of such techniques into tools for high-resolution cell monitoring and drug screening has, to the best of our knowledge, not been reported.

KEYWORDS: Single-Cell, Gold Nanoparticles, Nanotoxicity, High-throughput Assay

INTRODUCTION

Single-cell microfluidic approaches enable experimental statistical data to be acquired within a single device with a throughput comparable to that of standard flow cytometry, with the advantages of using reduced sample volumes and allowing for long-term monitoring of individual cells [1,2]. By bringing these features together with multiple optical imaging and spectroscopy techniques and enhanced signals from functionalized gold nanorods [3], intracellular activity and cell-nanoparticle interactions can be monitored in real-time and down to the single nanoparticle level, an area of increasing relevance with the growing use of nanomaterials [4]. Our proposed solution greatly improves upon previous work based on cells pre-incubated with gold nanoparticles [5] and offers new means to study cell-nanoparticle dynamics which are amenable to drug and vaccine delivery applications. A schematic representation of the system is presented in Figure 1.

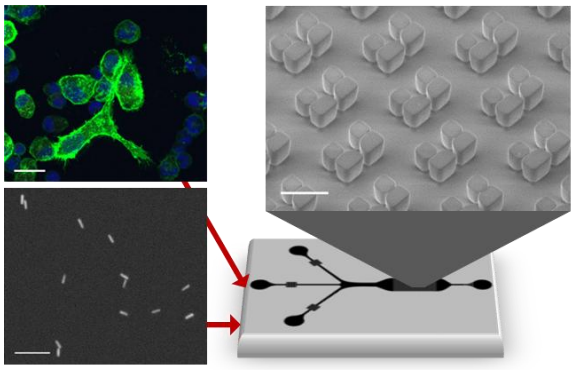

Figure 1: Schematic overview of the proposed system. Dendritic cells under confocal fluorescence microscopy (membrane staining with Cholera-toxin subunit B FITC conjugate, in green, and nucleus staining with DAPI, in blue). Scale bar is 20 μm. SEM image of nanorods with longitudinal λ_{max} of 765nm. Scale bar is 200 nm. Schematic of the microfluidic device and SEM image of the PDMS microtrap array. Channels are 25 μm deep and trap interior is 20 μm wide. Scale bar is 40 μm.

EXPERIMENTAL

A microfluidic device that enables trapping of a large number of single-cells in an array format was fabricated [1] using conventional soft-lithography methods [6] (Figure 1). Gold nanorod optical probes were produced through our adaptation [7] of an established seed-mediated synthesis approach [8],

followed by coating with Raman-active molecules, polyelectrolyte layers and proteins. Our custom-designed gold nanorods are visible via multiple optical imaging techniques (e.g. fluorescence microscopy and surface-enhanced Raman spectroscopy, SERS), with versatile optical properties and surface chemistry that can be fine-tuned for specific intracellular tracking and delivery applications. Primary (bone marrow-derived) dendritic cells were used, bringing additional challenges to cell trapping when compared to work done with cell lines [1,2], but allowing for more reliable assessment of cell function and heterogeneity.

RESULTS AND DISCUSSION

Cells were successfully trapped in the microfluidic device, which was kept in a microscope stage incubator to guarantee biocompatible conditions during these experiments (up to 24 hours). Controlled dispensing of nanoparticles onto the cell array in a gradient format was achieved (Figure 2a), with subsequent real-time monitoring of the cell response to different nanoparticle concentrations. Imaging of trapped cells and assessment of nanoparticle uptake was accomplished using bright-field and fluorescence microscopy and SERS (Figure 2b-c).

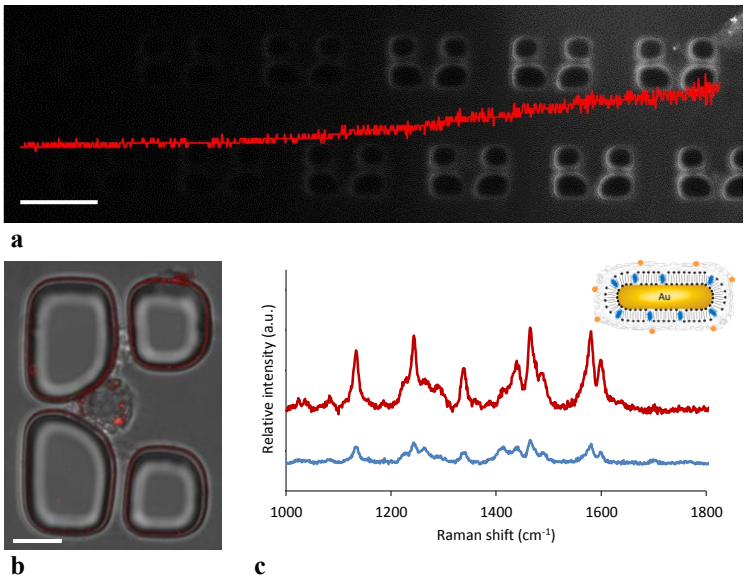

Figure 2: Controlled delivery of nanoparticles to trapped cells. a) Fluorescence image obtained when generating a concentration gradient of nanoparticles across the width of the trap array. The intensity profile shows increasing nanoparticle fluorescence at 633 nm excitation. Scale bar is 50 µm. b) Confocal bright-field image of a trapped dendritic cell. Intracellular nanoparticle identification is shown in red. Scale bar is 10 µm. c) Raman signal from PDMS chamber background (blue) and the trapped cell (red), showing notably higher intensity for specific nanorod signal within the cell. The figure insert is a schematic of the polymer-wrapped nanorod-dye conjugate. Spectra have been vertically offset for clarity.

Viability dyes were successfully used in long-term experiments to detect apoptotic (Annexin-V FITC) and necrotic (Sytox Blue) cell behaviour in response to the nanoparticles. Figure 3 shows an area of trapped cells and an example of the fluorescence information extracted in real-time for each trap. A certain degree of non-specific adsorption of the nanorods to the PDMS walls was observed, an issue that needs further investigation to define the minimal resolution of nanoparticle detection. Off-chip evaluation of cell-nanoparticle interactions (cell viability, nanoparticle uptake and activation status) was performed by incubating cells with nanorods and using flow cytometry and confocal microscopy.

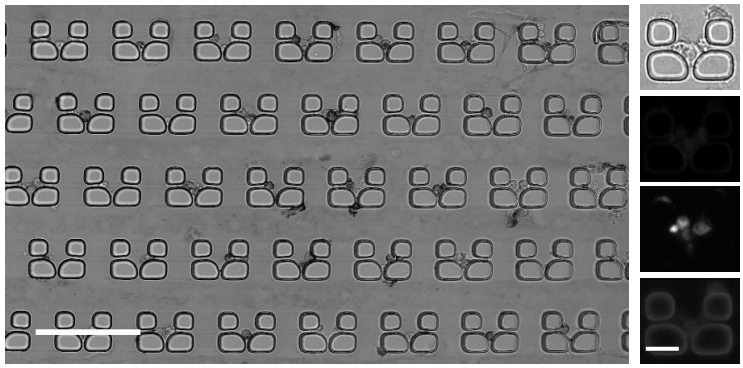

Figure 3: Real-time assessment of nanoparticle toxicity in trapped cells. a) Field of view of the microtrap array with trapped cells. Average single/double-cell coverage was approximately 80%. Scale bar is 100 μm. b) Microscopy images of nanoparticle uptake and cell viability, from top to bottom: bright-field image, necrosis marker (Sytox Blue), apoptosis marker (Annexin-V FITC) and gold nanorod fluorescence signals. Scale bar is 20 μm.

CONCLUSION

Results show proof-of-concept of our system for real-time, high-throughput testing of nanomaterial cytotoxicity, offering also the opportunity to assess nanoparticle uptake at the single-cell level. Current work is focused on understanding the cellular response to different formulations and concentrations of nanorods. By bringing together high-resolution imaging techniques, microfabrication techniques and nanoparticle probes, we aim to provide new insight into the immunological behaviour of dendritic cells related to intracellular, nanoparticle-mediated vaccine delivery.

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