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# A MICROFLUIDICS TOOL FOR HIGH-THROUGHPUT, REAL-TIME MULTIMODAL IMAGING OF NANOPARTICLE-CELL INTERACTIONS

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## KEY WORDS

single-cell, gold nanoparticles, nanotoxicity, high-throughput assay

## ABSTRACT

The increasing use of nanomaterials for biomedical applications has raised the need for efficient, robust and low-cost high-throughput assessment of nanotoxicity and cell-nanoparticle interactions. Microfluidics provides the tools for high-throughput single-cell functional monitoring, while gold nanorods have unique potential for intracellular tracking and can simultaneously be used as drug carriers. Presented here is a miniaturised platform that integrates these features with a multimodal approach to cell imaging. A microfluidic device allows for trapping of an array of single-cells, followed by the controlled delivery of nanoparticles into the cell array and subsequent real-time multimodal imaging of cellular interactions with functionalised nanoparticles. This system has been successfully used to assess cell-nanoparticle interactions at the single-cell level.

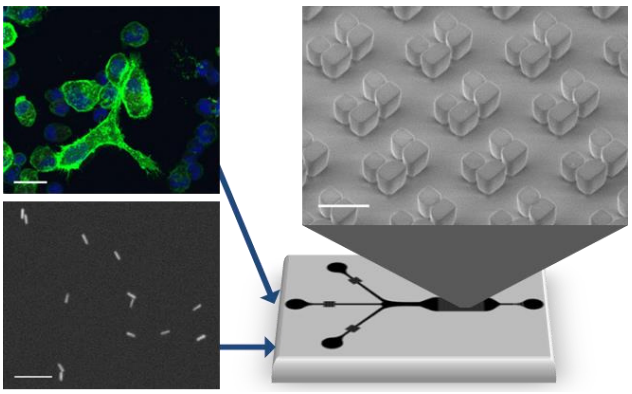

**Figure 1**: Schematic overview of the proposed system. Dendritic cells under confocal fluorescence microscopy (membrane staining with cholera-toxin-B, in green, and nucleus staining with DAPI, in blue). Scale bar is 20µm. SEM image of nanorods with longitudinal $\lambda_{max}$ of 765nm. Scale bar is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

## 1. 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

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nanomaterials [4]. This 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 Fig.1.

## 2. 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] (Fig.1). The device is compatible with various analytical methods, namely bright-field and fluorescence microscopy, confocal microscopy and surface enhanced Raman scattering (SERS) spectroscopy. Gold nanorod optical probes were produced through an adaptation [7] of an established seed-mediated synthesis approach [8], followed by coating with Raman-active molecules, polyelectrolyte layers and proteins. These custom-designed gold nanorods are visible via multiple optical imaging techniques (e.g. fluorescence microscopy and SERS), with versatile optical properties and surface chemistry that can be fine-tuned for specific intracellular tracking and delivery applications [9]. Primary (bone marrow-derived) dendritic cells were used, which brings additional challenges to cell trapping when compared to work done with cell lines [1,2], but allows for a more reliable assessment of cell function and heterogeneity.

Cells were trapped in the microfluidic device, which was kept in a microscope stage incubator with constant medium perfusion to guarantee biocompatible conditions for the duration of the experiments (up to 24 hours). A gradient of nanoparticle concentrations was delivered onto the array of trapped cells with subsequent real-time monitoring of the cell response to different nanoparticle concentrations (cell motility and fluorescent markers of apoptosis and necrosis), using bright-field and fluorescence microscopy and SERS. Trapped cells were also fixed within the device for further analysis. As a control experiment, 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.

## 3. RESULTS AND DISCUSSION

### 3.1 Microfluidic cell trapping and controlled nanoparticle delivery

Cells were successfully trapped and kept alive within the microfluidic environment. Controlled dispensing of nanoparticles onto the cell array in a gradient format was achieved (Fig.2a). Multimodal imaging of trapped cells (bright-field, fluorescence microscopy and SERS) and assessment of nanoparticle uptake was accomplished, an example of which is shown in Fig.2b-c.

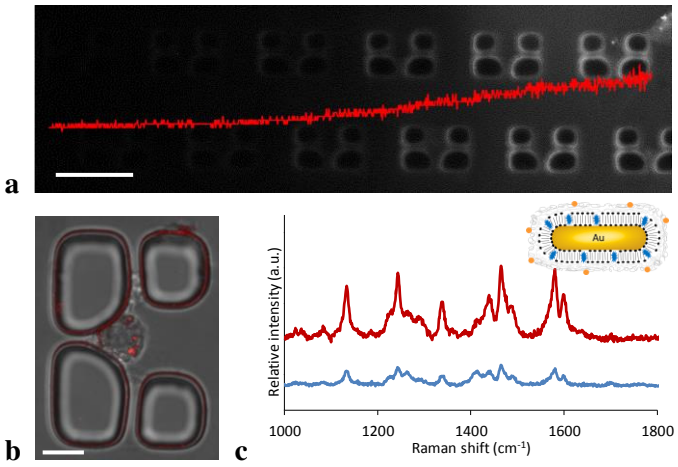
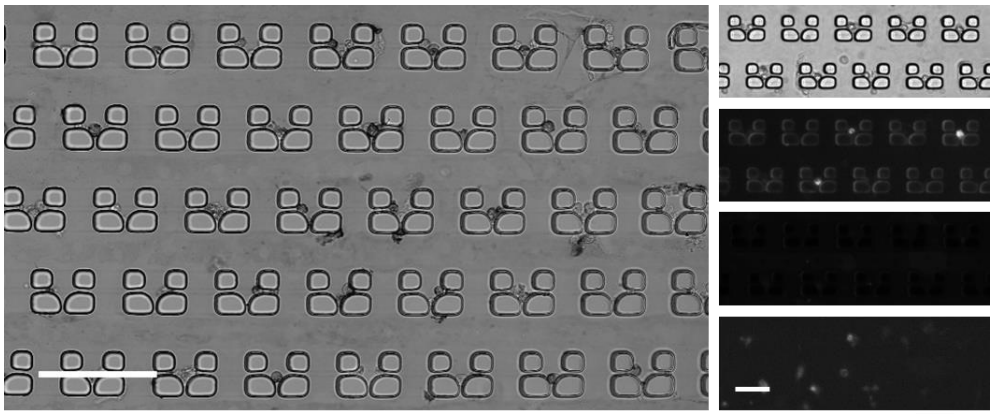



**Figure 2**: Controlled delivery of nanoparticles to trapped cells. a) Fluorescence image obtained when generating a concentration gradient of nanoparticles across the width of the trap array. The intensity profile shows increasing nanoparticle fluorescence at 633 nm excitation. Scale bar is 50 µm. 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.

### 3.2 High-throughput assessment of cell-nanoparticle interactions in the microfluidic device

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. Fig.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.



a b

**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, gold nanorod fluorescence (633 nm excitation), necrosis marker (Sytox Blue), apoptosis marker (Annexin-V FITC). Scale bar is 50 µm.

Uptake of nanorods by dendritic cells within the microfluidic array was qualitatively estimated by monitoring the increase in nanorod-specific fluorescence intensity during nanoparticle delivery (Fig.4). Results show a clear difference in this increase between the tracked cells and the background, indicating that the cells were taking up the nanorods. The initial delay of the fluorescent signal (0-15 minutes) is due to a lag time between activation of the flow of nanorods and their presence in the array due to the compliance of the system (syringe-PDMS). Future work includes, on one side, relating different degrees of uptake to cell motility and activation status, and on the other side, enhancing control of uptake by manipulating the nanorod surface chemistry, ultimately aiming at intracellular targeting and single-nanorod tracking.

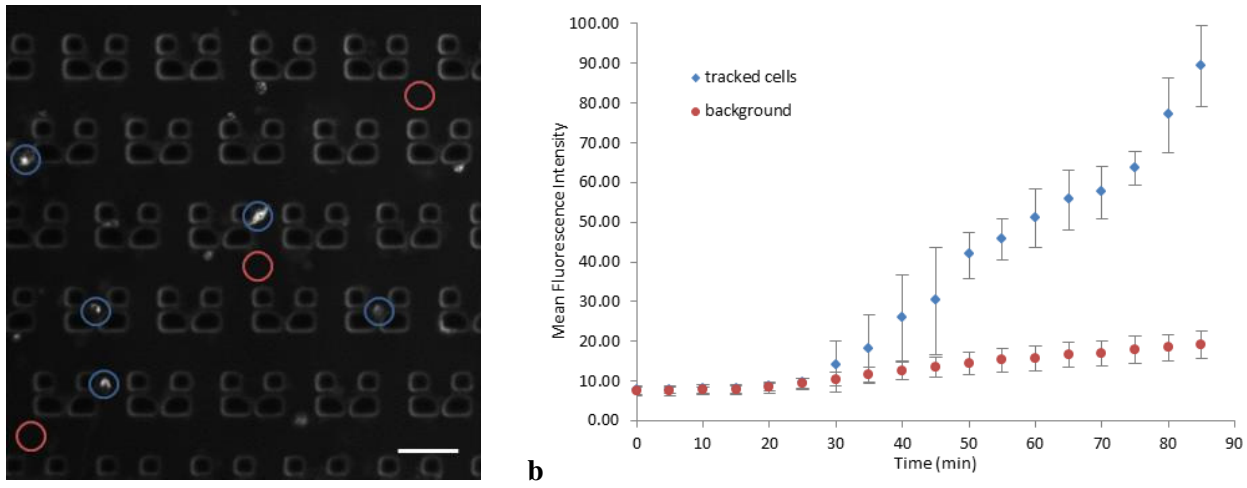



**Figure 4**: Qualitative estimation of nanorod uptake by dendritic cells in the microfluidic device. a) Representative frame from a video obtained during nanoparticle delivery to cells in the microfluidic array, showing the fluorescence intensity signal of five tracked cells (blue circles) and three tracked background areas (red circles) during nanoparticle delivery. Scale bar = 50 µm. b) Temporal progression of the average MFI value of the three background regions and the five cells tracked from Fig.4a. Error bars represent standard deviation.

Assessment of cell-nanoparticle interactions performed off-chip using flow cytometry and confocal microscopy was successful in the detection of nanoparticle uptake for different concentrations of nanoparticles and times of exposure (Fig.4). Results obtained on and off-chip showed no significant toxicity of the nanoparticles at the concentrations and exposure times used, and each microfluidic device was capable of providing large sets of data (around 400 cells analysed in only half of the trap array). This shows the potential of this tool to work simultaneously for high-throughput and high-resolution cell monitoring, with the added feature of allowing for dynamic real-time imaging of individual cells, as opposed to population end-point measurements as obtained with flow cytometry.

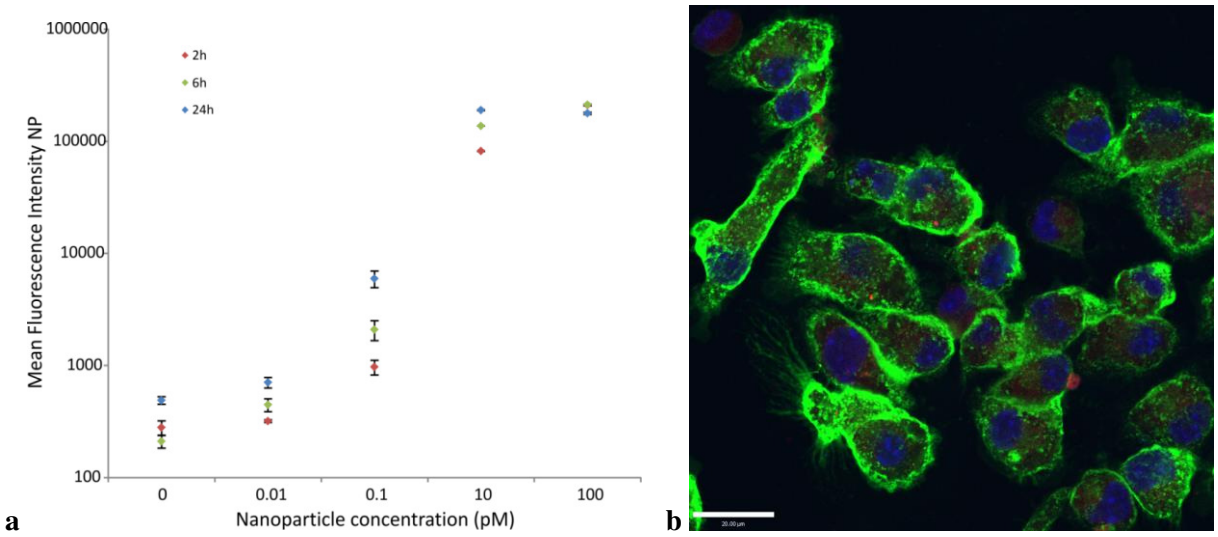



**Figure 5**: Nanoparticle uptake by dendritic cells measured using flow cytometry and confocal fluorescence microscopy. a) Cells were incubated in static conditions with different nanoparticle concentrations in duplicates and the fluorescence intensity at 633 nm excitation was obtained using a flow cytometer at different time points. The graph shows the average of the two samples for each condition, with error bars representing standard deviation. b) Confocal fluorescence image of dendritic cells stained with cholera-toxin-B (membrane, in green) and DAPI (nucleus, in blue) with nanoparticle fluorescence at 633 nm excitation shown in red. Scale bar = 20 μm.

## 4. CONCLUSION

Results show proof-of-concept of an integrated system for real-time, high-throughput testing of nanomaterial cytotoxicity, which is amenable to different imaging methods and offers 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, specifically through analysis of cell motility and viability. By bringing together high-resolution imaging techniques, high-throughput microfluidics and highly specific nanoparticle probes, this work aims to provide new insight into the immunological behaviour of dendritic cells related to intracellular, nanoparticle-mediated vaccine delivery, as well as providing a versatile tool with numerous cell monitoring, drug screening and nanomaterial testing applications.

## ACKNOWLEDGEMENTS

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