# TIME-LAPSE MEASUREMENT OF SINGLE-CELL RESPONSE TO NANOMATERIAL: A MICROFLUIDIC APPROACH

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## ABSTRACT

This work presents the successful application of a single-cell microfluidic platform for high-throughput, real-time screening of nanoparticle-cell interactions. Taking vaccine delivery as a proof-of-concept application, ovalbumin-conjugated gold nanorods were produced and controllably delivered to primary dendritic cells within the device. Time-lapse imaging enabled monitoring of hundreds of single-cells during exposure to a range of concentrations of nanoparticle conjugates and simultaneous quantification of specific cellular functions. This integrated system provides throughput and statistical data comparable to that obtained with flow cytometry but also offers a novel approach to determine the dynamics of nanoparticle-cell interactions and nanoparticle-mediated antigen delivery with single-cell resolution.

**KEYWORDS:** Single-cell, gold nanorod, nano-vaccine, high-throughput assay

## INTRODUCTION

Nanomaterials are increasingly being investigated for biotechnology, diagnostic and therapeutic applications, including vaccine delivery [1], with gold nanoparticles have demonstrated potential as intracellular optical probes [2], as well as vaccine carrier candidates due to their biocompatibility and potent adjuvant ability. Dendritic cells (DCs) are a privileged target for vaccine delivery due to their role in the initiation of the immune response [3]. Also, successful targeting to DCs would allow for increased stimulation at lower antigen dose, thus reducing side-effects and vaccine cost [3]. Microfluidics has shown great promise for providing tools to investigate immunological functions and cell-nanoparticle interactions, but challenges still remain to create tools that enable the effective evaluation of the dynamics and heterogeneity of these interactions [4] and, importantly, those involved in the intracellular processing of antigen via nanoparticle mediated delivery [3].

## EXPERIMENTAL

Microfluidic devices were fabricated using standard soft- and photo-lithography procedures and consisted of three inlets, a hydrodynamic trapping chamber containing an array of over 1,500 traps, and one outlet (Fig.1). Each trap had an inner width of 20 µm and constituted a low shear stress pocket (< 0.05 dyn/cm$^2$) that induced cell trapping without damaging cell membrane [5]. Primary, murine bone marrow-derived DCs were used in all experiments. Gold nanorods (NR) were synthesised and functionalised into highly stable and specific optical probes, as previously described [2]. Ovalbumin (OVA) was used as a model antigen, with NR-OVA conjugates constituting a system to induce a trackable immune response in primary DCs. DQ Ovalbumin (DQOVA) is an ovalbumin conjugate containing a quenched dye which becomes fluorescent upon cleavage by intracellular proteases, indicating antigen processing by DCs. A gradient of NR-DQOVA concentrations was controllably delivered to the trapped cells, while NR uptake and antigen processing were monitored and recorded in real-time from >500 single-cells over 8 hours.

## RESULTS AND DISCUSSION

Cells were successfully trapped within the microfluidic chamber, with typically ~83% of the traps containing between 1 and 3 cells. Subsequently, single-cell monitoring was achieved by tracking the response of each cell individually. This way, using bright-field and fluorescence microscopy time-lapse imaging, the microfluidic platform enabled hundreds of single-cells monitored in real-time during controlled exposure to a range of concentrations of NR-DQOVA conjugates. Specific cellular functions could then be quantified, including nanoparticle uptake and antigen processing, based on the fluorescence intensities of individual cells (Fig.1). The results show the capabilities of the developed platform to provide statistically relevant population analysis with single-cell resolution and represent novel information about the dynamics of the interaction of nanoparticles with single-cells and, specifically, nanoparticle-mediated antigen delivery.

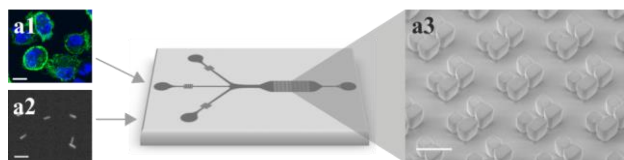


*Figure 1. Schematic representation of the microfluidic system and procedures developed. Dendritic cells (a1, scale bar = 10 µm) and nanorods (a2, scale bar = 100 nm) were sequentially injected into a microfluidic device containing an array of cell microtraps (a3, scale bar = 40 µm).*

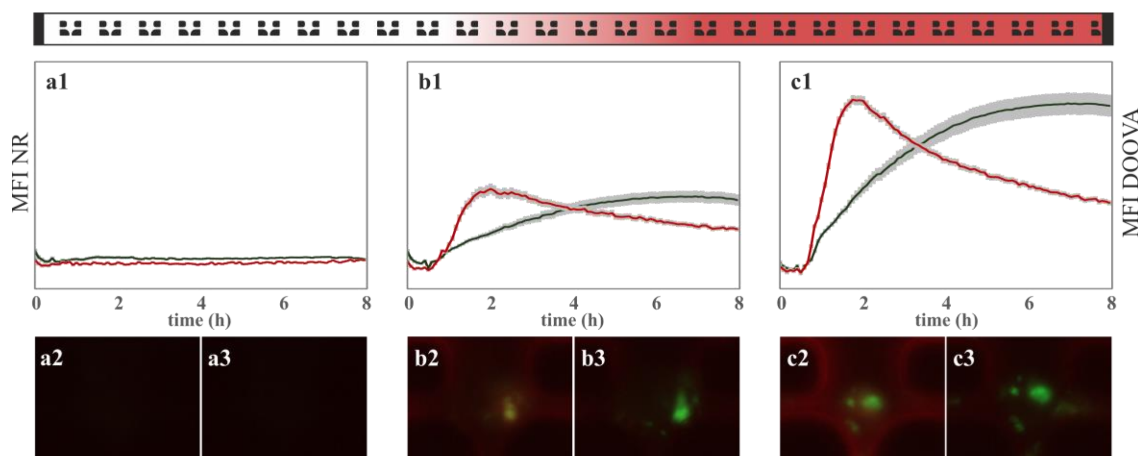


*Figure 2. Population and single-cell analysis following nanorod uptake and antigen processing. Circular ROIs were drawn around individual cells for data acquired every 5 minutes over 8 hours. For analysis purposes, three areas were defined according to different nanoparticle concentrations: (a) Null (N=151), (b) Medium (N=203) and (c) High concentration (N=171) regions, as per the illustrated nanoparticle delivery profile. Graphs show the response of single cells in each of these regions. (a1-c1) Average NR fluorescence intensity (633 nm excitation) and DQOVA fluorescence (488 nm excitation) ±S.E.M. (background corrected) (a2-c2) Composite fluorescence images of examples of single-cell ROIs in each region at 2 hours and (a3-c3) 6 hours, where red shows NR fluorescence (633 nm excitation) and green shows DQOVA fluorescence (488 nm excitation).*

## CONCLUSION

Overall, the platform presented here integrates microfluidic, nanomaterial and imaging techniques to provide a new methodology for investigating the dynamics involved in the intracellular processing pathways regarding antigen delivery from the surface of nanoparticles. This system can be adapted for many nanomaterial formulations and cell types, making it a versatile tool for the screening and development of nanomaterials for biomedical applications.


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