Discovery-Q: a whole cell biosensor used to evaluate the mechanism of phagocytosis of macrophages using different particles.

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Technology

We have developed a multi-well cell-based sensor that can monitor real-time biological changes in living cells, such as mass redistribution, and viscoelasticity. This system provides unique kinetic information regarding the phenotypic change in the cells post treatment (Fig 1).

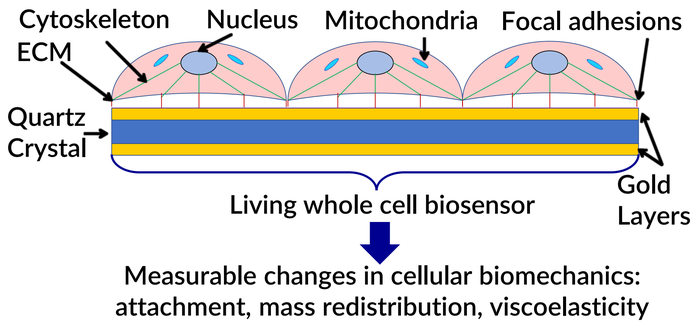

Figure 1. Quartz Crystal Microbalance method of detection

Our biosensor allows the investigation of downstream signaling in whole living cells maintained in culture conditions. The experiments are conducted with the same technique as a cell culture assay that take place in a cell culture well plate (Fig 2). This environment and method allows the cells to respond to the drug that is added. Integrated in each well is a piezoelectric sensor that the cells adhere too (Fig 1). This enables us to measure in real time the changes that occur within a cell in response to drugs or agent.

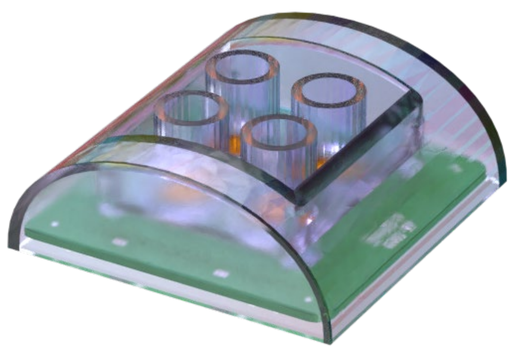

Figure 2. Discovery-Q Device, in incubator, with humidity, temperature and CO_2 control

Introduction

Targeted drug delivery to macrophages appears to be growing in interest and popularity as a way to improve therapeutic drug delivery and efficiency.

The development of drug delivery systems has reached many roadblocks such as *in vivo* stability, drug solubility, efficient targeting, cytotoxicity, as well as targeted receptor mediated uptake of drugs/particles. Macrophages engulf a variety of different targets including pathogens, and foreign particles, and the target properties influence their uptake and response. Understanding how macrophages respond to carriers or targets as well as which receptor-mediated phagocytosis is initiated is vital when developing particles for drug delivery.

As a proof of principle study, we evaluate DH82 macrophage phagocytosis using three different particles: latex beads, Zymosan A and *Staphylococcus aureus*. These studies show the Discovery-Q's ability to distinguish and differentiate the unique physiological method of macrophage phagocytosis. In this case, three different types of particles are tested: indigestible particles (latex beads); and digestible particles of different origins – Zymosan A (yeast cell wall) and *S. aureus* (heat killed wood strain bacteria).

A macrophage will respond differently when it encounters particles it can't digest, or biological material like yeast and bacteria which pose infection risks. Each particle triggers a unique receptor on the macrophage to initiate phagocytosis and dictates how the particle will be internalized.

Methods

125,000 DH82 cells-per-well were plated into the Discovery-Q system. After a period to allow for cell adhesion, the media was refreshed. After the cells stabilized (XX hours), a baseline frequency (f) and resistance (R) were obtained.

At that point, the three types of particles were introduced, and the traces were acquired. The 0.8 µm latex beads (0, 1:100, and 1:230 cells-per-particle), pHrodo™ Red Zymosan A BioParticles® Conjugate (0, 1:8, and 1:16 cells-per-particle), and *pHrodo™* Green *S. aureus* BioParticles® Conjugate (0, 1:5, and 1:25 cells-per-particle). Using the change in frequency (Δf) and change in resistance (ΔR) data acquired over 24 hours, times of interest were selected that represent phagocytosis and regurgitation (Fig 3).

The experiments were replicated in Lab-Tek II culture slides at the time points of interest selected from the traces. Using fluorescent imaging, the mechanisms of action were captured (Fig 4). In the latex beads portion of the experiment, the cytoplasm was stained with LavaCell (red) and the nucleus with DAPI (blue). The pHRodo Zymosan A bioparticles were red; the cytoplasm was stained with CellTracker™ (green) and the nucleus with DAPI (blue). The *S. aureus* BioParticles were green; the cytoplasm was stained with LavaCell (red) and the nucleus with DAPI (blue).

Discussion

Phagocytosis requires complex structural rearrangement and signaling allowing for clearance of infectious agents, as well as inorganic particles. The process involves three stages: recognition by receptors; receptor mediated internalization and cleavage of phagosome; and intercellular trafficking ultimately leading to degradation.

Latex beads have been studied for their phagocytic activity as non-degradable particles, and they have been characterized as a good model to analyze. Phagocytosis of latex beads has been linked to scavenger receptors. In the first 30 minutes of the macrophage traces with the latex beads, there is no significant Δf or ΔR (Fig 3A). By 3.5 hr, the Δf increases; this correlates with the images of that time showing phagosomes indicated by the white arrows (Fig 4). The increase of phagocytosis continues until 10 hours where increased phagosomes are visible (Fig 4). However, the 230 particles to 1 cell concentration continues to perform phagocytosis, which can be seen by the deviation of the traces at that point (Fig 3A). Microscopy alone cannot quantify that change.

For the two biological specimens, pHrodo™ conjugated particles were obtained. The pHrodo™ dye increases in fluorescence when the pH becomes more acidic. These particles are non-fluorescent outside the cell, but fluorescent in the phagosomes. Zymosan A particles have been used as a model for pathogen associated recognition of microbes by the innate immune system, inducing inflammatory signals and mechanisms of phagocytosis recognized by Toll-Like Receptor 2 (TLR2) in macrophages. With the Zymosan A traces, and immediate response to the particles can be seen by the sharp increase Δf and corresponding decline in ΔR (Fig 3B). This response can be visualized by the red Zymosan A particles – they are only fluorescent when undergoing phagocytosis (arrow, Fig 4). As the macrophages continue to engulf the particles, there is a steady decrease in Δf, which corresponds to spreading of the cells (Fig 3B). This can be visualized by the increase in Zymosan A particles within the macrophages and the increased cytoplasmic area (arrow, Fig 4).

Results

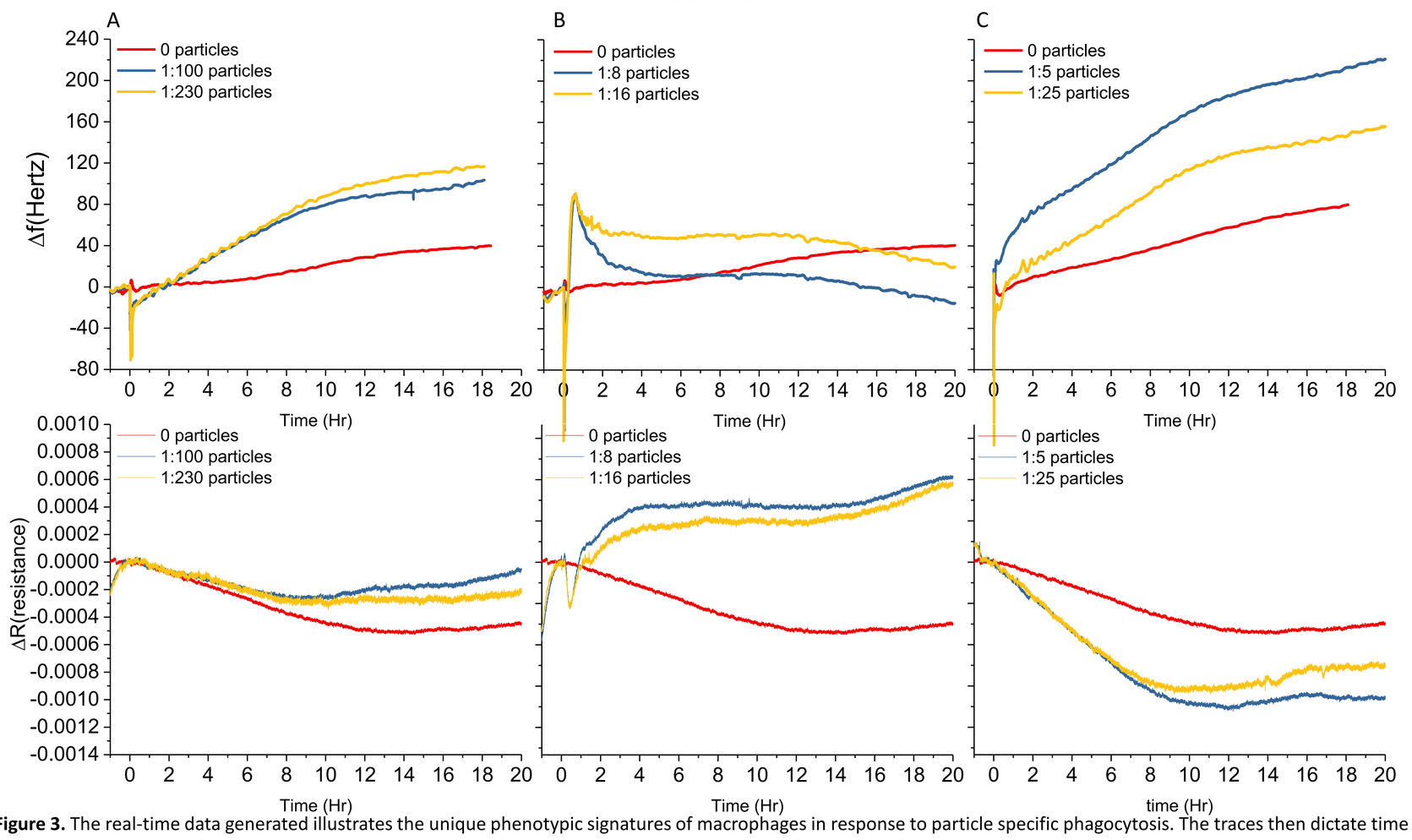

Figure 3. The real-time data generated illustrates the unique phenotypic signatures of macrophages in response to particle specific phagocytosis. The traces then dictate time points at which visualization should occur, and guides the elucidation of the mechanism of action. The top row is change of frequency (Δf), and the bottom row is change of resistance (ΔR). A) latex bead, B) Zymosan A C) S. aureus

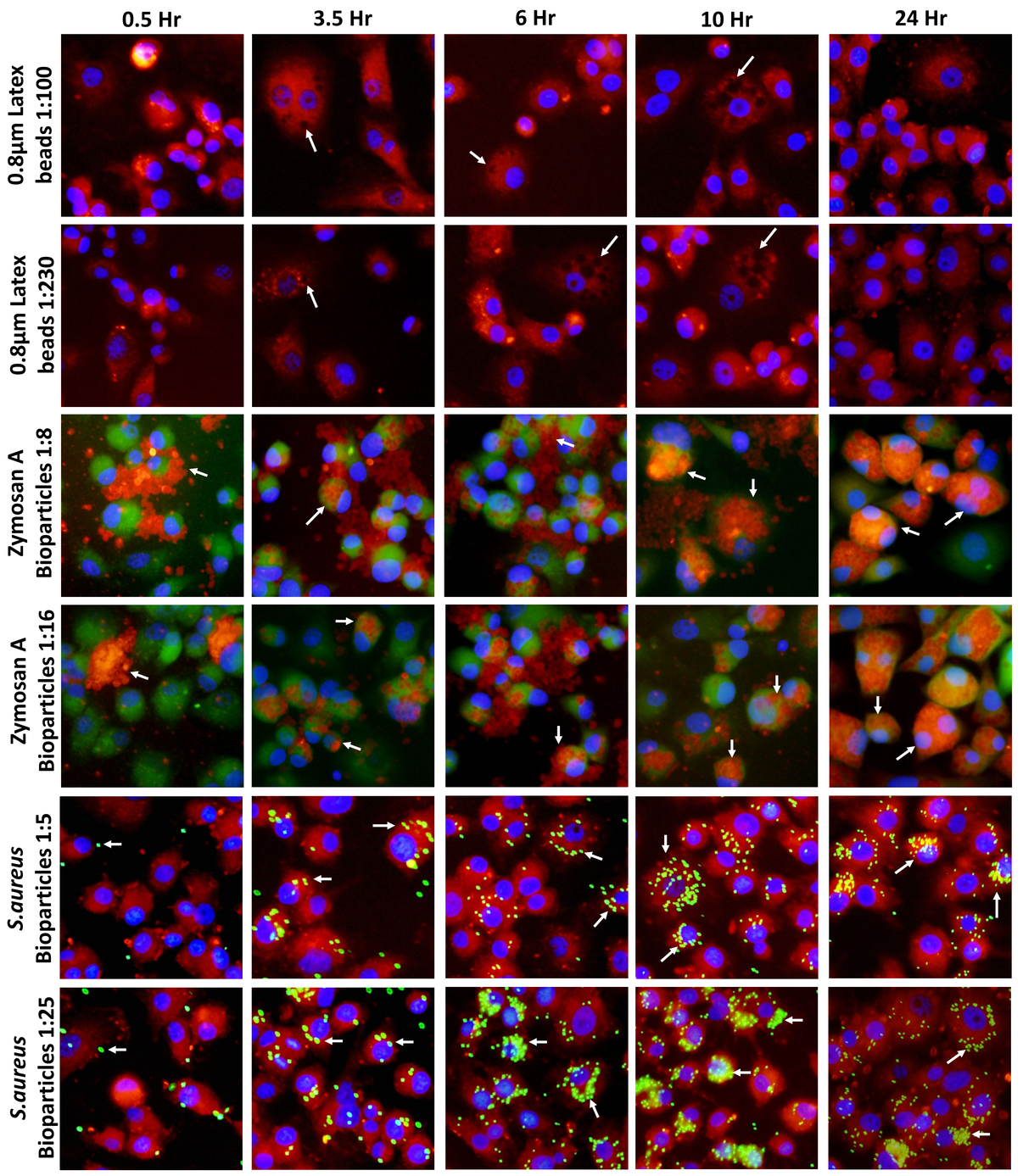

Figure 4. Fluorescent imaging of macrophage cells performing phagocytosis with three different particles, an indigestible particle (latex beads), and digestible particles (Red Zymosan A (yeast cell wall) and Green *S. aureus* (bacteria)).

When the macrophages come into contact with *S. aureus*, their TLR is activated. This version of inactivated *S. aureus* cannot initiate mechanisms. The contact of the *S. aureus* particles to the macrophages – like the Zymosan A – triggers an immediate increase in the Δf (Fig 3C). However, the initial magnitude is less than that of Zymosan A, and can be verified with the microscopy of the 30-minute time point (Fig 4). In contrast to Zymosan A, *S. aureus* continues to exhibit a rise in Δf and a drop in ΔR (Fig 3C). By 10 hr the macrophages increase the rate of phagocytosis as seen in Figure 4, but unlike with Zymosan A, where the Δf drops (Fig 3B), the Δf of *S. aureus* rises. Microscopy shows that the macrophages have digested the particles, as seen by the reduction in the particle size (Fig 4). This suggests more effective phagocytosis has been performed. Following the traces for a longer period would determine if the particles are fully digested and the cells return to a new baseline, or if they undergo controlled cell death as a result of the phagocytosis.

Conclusion

Each particle type generates a unique trace. With this immune cell, if we introduced an unknown particle, by matching the trace to our known traces, we can determine which cell receptor was utilized for phagocytosis. This means that if a researcher wants to target a specific receptor, the drug or particle needs to elicit the correct trace. If the researcher wants to avoid phagocytosis, for example with a targeted treatment particle, then the drug or particle needs to avoid generating phagocytosis traces, because this would destroy the drug vs. deliver it to the cells being treated.

In drug discovery, binding kinetics experiments and flow cytometry experiments prove that a molecule binds a target. However, the researcher does not know which of the candidates will elicit the strongest response in a cell, or avoid triggering an unwanted response. Using our system, researchers can determine when a response is elicited, and what that response elicits, elucidating the mechanism of action.