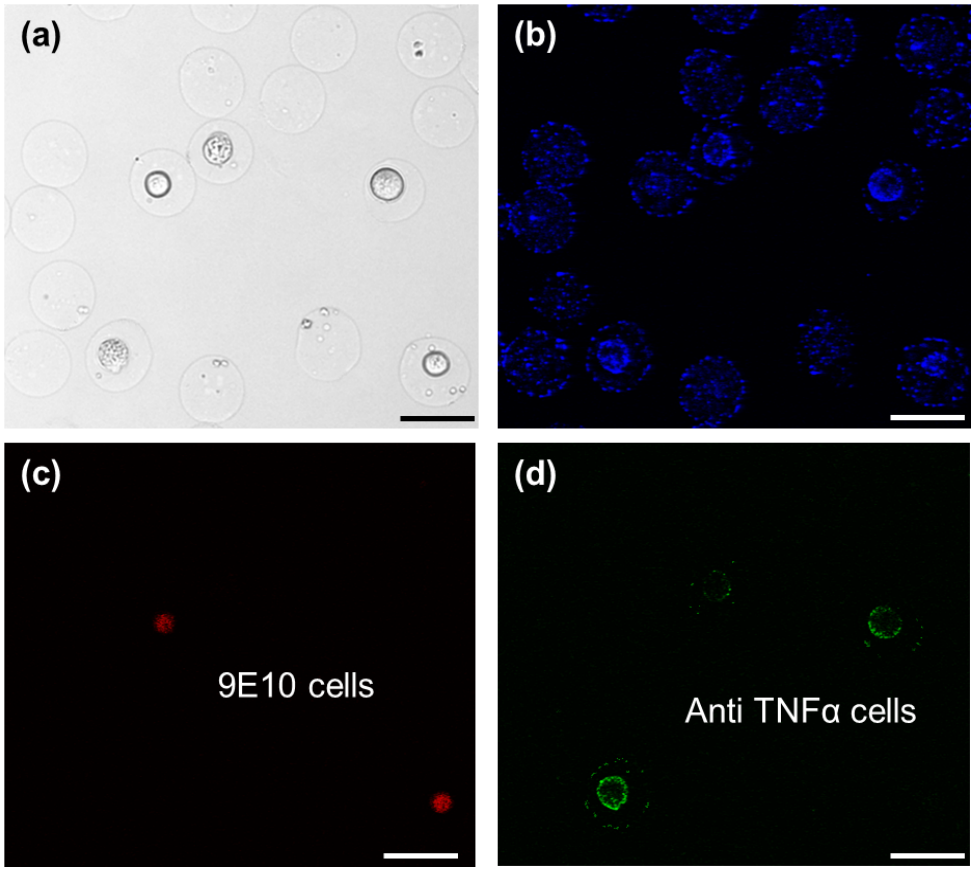
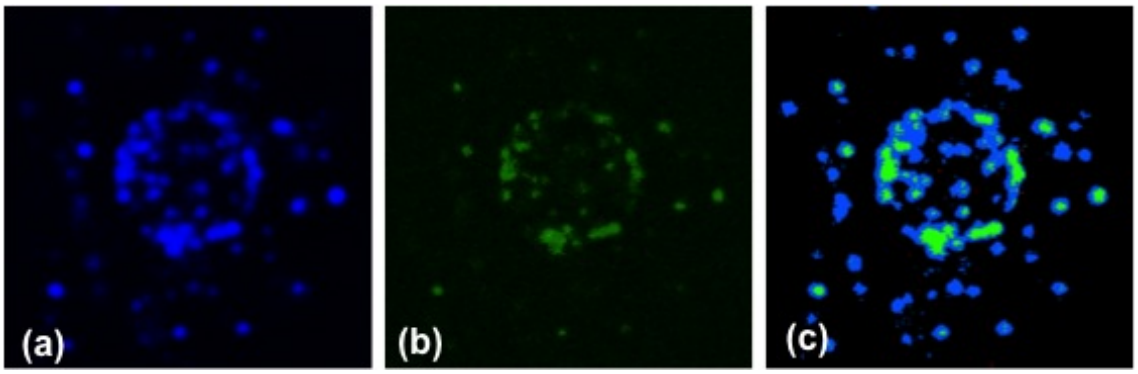
Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary material



Supplementary figure 1- (a) Bright field image of alginate microparticles transferred to cell growth medium after incubation for 1.5 hours. (b) The IgG capturing complexes are visualized in the far red channel due to the Alexa flour ® 647 conjugated donkey anti-rabbit antibodies in the complex. The high molecular weight capturing complexes are retained in the particles after washing and transferring to cell medium. (c) The 9E10 cells are are pre-stained by orange live dye and are distinguishable from anti-TNF-α antibody secreting cells. Two particles contain anti-myc antibody secreting cell (9E10) and three other particles encapsulate anti TNFα antibody-secreting cell. (h) The TNF-α antigens are completely washed away from the particles encapsulating the anti-myc antibody-secreting cell while they have bound to the IgG complexes in the particle encapsulating the anti TNFα antibody-secreting cell.



Supplementary Figure 2- (a) A microparticle encapsulating single anti TNFα antibody-secreting cell and high molecular weight capturing molecules visualized as blue in the far-red fluorescent channel. (b) The FITC conjugated TNFα antigen bound to the high molecular weight capturing IgG complexes are visualized in the green channel. (c) Co-localization of the TNFα antigens with the capturing complexes computed by Squaash software.

Materials and methods for the microfluidic based immunoassay

The microfluidic-based drop maker with channel dimensions of 20 μm in width and 25 μm in height was fabricated using soft lithography in polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning). The channels were coated with Aquapel (PPG Industries, Pittsburgh, PA) to become hydrophobic.

Sterile alginate Pronova SLG100 (Novamatrix, Norway) was dissolved in 10 ml of cell growth medium CO_2 independent DMEM (Gibco, Grand Island, NY) to prepare 2.5% w/w alginate solution. Based on the desired concentration of alginate microparticles, this solution was further diluted with cell growth medium and Optiprep (Axis-shield, Norway). Optiprep is included at 16% final concentration (v/v) to match the density of the alginate solution with that of the cells so that the cells remain suspended during particle-making. Anti-myc 9E10 cells are pre-stained with live dye orange cell tracker (Invitrogen) and mixed with anti-TNFα hybridoma cells in the alginate solution. Cell concentration is calculated to have only one cell in each droplet following the Poisson statistics. The cells, medium, alginate solution and syringes are all cooled on ice to minimize antibody secretion prior to drop generation. In the presented results, we have used two 1.5% w/w alginate solutions; one containing the cells and rabbit anti-goat Fcγ specific (Jackson immunoresearch, West groove, USA) and the other one with 70 mM calcium carbonate nanoparticles, goat anti-mouse (Fcγ specific), donkey anti-rabbit (heavy and light chain specific), and fluorescein conjugated TNF-α antigen. Concentrations of antibodies are calculated to have $4x10^6$ IgG molecules from each antibody in 40 μm diameter droplets. We used the same flow rate for both of the solutions resulting in droplets of 35 mM $CaCO_3$ and 1.5% w/w alginate. Fluorinated surfactant is solved in Novec 7500 flourocarbon oil (1% w/w) was used to stabilize the droplets in oil phase and prevent drop coalescence.

Prior to dropmaking, an acidic oil solution was prepared by dissolving 1µl glacial acetic acid (Sigma, St. Louis, MO) in 1 ml oil (Novec 7500, 3M, St Paul, Minnesota) resulting in 17.5 mM acidic oil solution. During dropmaking, droplets were collected every 20 minutes. The molar quantity of calcium carbonate in the collected drops was calculated according to its initial concentration and the flow rate of aqueous solution. One third of the stoichiometric equivalent of acetic acid with respect to the calcium carbonate content was dissolved in oil and added to the collected drops to partially polymerize and stabilize the droplets without decreasing the pH to harm the cells. The semi-gelled particles are then incubated for 1.5 hours at 37ºC and 5% CO_2 until enough antibodies are secreted. Then, the rest of the acetic acidic is added to completely polymerize the particles. The particles were pelleted by centrifuging at 200 rpm for 3 s and the oil phase was removed. Then, 1 ml of 20% v/v of PFO (1H,1H,2H,2H-Perfluoro-1-octanol 97%, Sigma-Aldrich, USA) in Novec 7500 oil was added to dissolve the surfactant from the particles. The mixture was centrifuged at 200 rpm for 3 s and the oil/PFO phase was removed. The particles were then washed twice with fresh cell growth medium (centrifuging for 30 sec at 300 rpm) to ensure that all acidic oil was removed. The particles re-suspended in fresh growth medium and used for microscopy.