

noise ones. **Method:** To address this issue we looked at the signal/noise ratio of some low abundance antigens on various cell lines under different sheath pressure from 10-70 PSI of BD FACS *Aria* Flow Cytometer. **Results:** Contrary to the considerable non-linear increase in mean fluorescence intensity of Fluorescent beads at lower pressures, the S/N ratio of the cells showed an optimum based trend. **Conclusion:** The bio-physical features of cells might have an impact on the mean fluorescent intensity area of the signals at different pressures which suggest a kind of pressure titration for obtaining the optimal detection of dimly expressed antigen

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CHARACTERIZATION OF AN ERBB2-TETOFF REPRESSIBLE CELL LINE AS A POSSIBLE MODEL FOR TESTING ERBB2 TARGETED THERAPIES

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ErbB2 is a receptor tyrosine kinase overexpressed and resulting in bad prognosis in several human malignancies. Trastuzumab (TR), a humanized anti-ErbB2 antibody is a specific targeted therapy against these tumors, with a history of both success and a high rate of therapy resistance. Models of therapy targeting are thus much needed to improve current modalities and to discover novel ones. NIH3T3 ErbB2-SA_DR is a derivative of NIH3T3 fibroblasts stably transfected with ectopic human ErbB2 under the control of a TEToff system that allows the repression of ErbB2 expression by tetracycline (Tet). We characterized the cell line in terms of ErbB expression, stability, repressibility by Tet and consecutive recovery. Furthermore, effect of TR treatment on proliferation, cell cycle, apoptosis, as well as ErbB2 expression and phosphorylation was investigated as a function of passage number (P).

Immunofluorescent labelling and flow cytometry showed that of the ErbB family, only the ectopic human ErbB2 was found in the cells, at a copy number of 700.000. 40 ng/ml tetracycline was sufficient for knocking down ErbB2 expression, below this, the response was dichotomic; some cells showed an almost complete downregulation, others were unaffected. It took 48 hrs for ErbB2 to disappear from the cell membrane, and over 3 weeks to reappear (and only in 40% of the population). With increasing P, overall ErbB2 expression decreased somewhat, and Tet downregulated ErbB2 to a lesser extent at the single cell level (but same overall effectiveness).

Initially, cells were not sensitive to proliferation inhibition by 100ug/ml TR over 3 days (as determined by an MTT based assay). However, at the P11, sensitivity appeared and increased to P20. PI-based flow cytometric cell cycle determination showed a 10-15% G1 block both upon TR (coherent with earlier findings for ErbB2 positive tumors) and also upon Tet, the two not being additive, confirming specific TR action via ErbB2. There was no sub-G1 fraction in any of the samples and there was a stable ~1% Annexin-V positivity in any P under any treatment. However, both TR and Tet increased the PI positive necrotic population from 0.5 to 2.5%, which is contrary to the known pro-apoptotic effect of TR in many human tumors.

Cells double immunolabelled for ErbB2 and phospho-ErbB2 show that 3 day TR downregulates the ectopic ErbB2 only in later Ps, and progressively boosts a low-ErbB2 subpopulation. In line with this, a 5 min TR treatment cannot enhance ErbB2 phosphorylation in early Ps, but promotes P-ErbB2 levels in P11 and more in P20. Blocking phosphatases with vanadate increases P-ErbB2 only mildly in P3, but far more in later Ps. It is likely that the complete signalling and regulatory system around the ectopic ErbB2 evolves in time, including the progression to a more efficient ErbB2 phosphorylation, higher rate of dephosphorylation and turnover, and eventually addiction to the oncogene and sensitivity to its targeting.

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ELASTOMERIC MICROPARTICLES THAT EXHIBIT NEGATIVE ACOUSTIC CONTRAST IN BIOASSAYS

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Introduction: The development of more sensitive and rapid medical assays is imperative to decreasing time-to-diagnosis in diseased state individuals, and thus to improving patient outcomes. Enhanced detection limits afford the ability to detect the presence of low concentrations of biomarkers that may be present during the early-onset stages of a disease. Reduction in sample preparation requirements can further decrease assay time and the expertise level of the care provider. Our research aims to develop a sensitive and rapid bioassay platform using elastomeric capture microparticles (ECμPs) coupled to acoustic flow cytometry. ECμPs possess unique physical and mechanical properties enabling the separation of ligand-bound ECμPs from biological particles (e.g., red blood cells, white blood cells, and plasma proteins) within an unmodified matrix (e.g., whole blood) by placing them under acoustic pressure. The use of ECμPs in recently developed low cost acoustic flow cytometry instrumentation will have significant benefits. ECμPs have acoustic properties (negative contrast) that allow positioning at different acoustic pressure potential minima than biological particles (positive contrast) within an acoustic pressure field. Hence, acoustic pressure within an acoustic flow cytometer can be used as a binary separation method for removing unwanted biological particles from ligand-bound ECμPs within biological samples. This removal of unwanted biological particles will increase the signal-to-noise ratio and thus result in increased sensitivity. It will also decrease sample preparation time. We anticipate that our system will constitute an advance in sensitivity, specificity, and assay speed over existing systems.

Methods: Monodisperse elastomeric droplets were synthesized using microfluidic devices. These devices were used to emulsify elastomer prepolymer and crosslinking agent within an aqueous phase containing a stabilizing surfactant to form monodisperse elastomeric droplets. Droplets were then cured overnight to form crosslinked negative contrast elastomeric microparticles (ECμPs). ECμPs were then functionalized by methods to provide biomolecular specificity. In a proof-of-concept, our negative contrast ECμPs were mixed with positive contrast polystyrene beads and injected into an acoustic focusing chamber driven by a piezoelectric driver. Specific acoustic frequencies were then used to separate negative and positive contrast particles. **Results:** We have successfully synthesized monodisperse ECμPs using microfluidic devices that exhibit negative contrast in acoustic fields. These ECμPs can be easily separated from positive contrast polystyrene beads using acoustic radiation within acoustic focusing chambers. We have demonstrated methods for biofunctionalizing these ECμPs for biospecific interaction analysis. We are currently investigating the application and performance of these particles in assays performed by acoustic flow cytometry