IN *SITU* MICROFLUIDIC BIOFUNCTIONALISATION TO FORM MULTIVALENT INTERACTIONS AND INVESTIGATE CELL ROLLING AND PHENOTYPE MODIFICATION

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ABSTRACT
We propose a method to biofunctionalise microfluidic systems for cellomic investigations based on microfluidic protocols. The biofunctionalised devices were used to perform cell rolling and investigate tumor cell phenotype changes. The strong attention to the modification of the environment by biomolecules (antibodies, proteins or carbohydrate and lectins) has encouraged the studies of the cell behaviour in biomimetic environments. The final aim of cell rolling investigations is to give etiological explanation of diseases, pathologies and sudden change in cell behaviour or cell phenotype and genotype.

KEYWORDS
Biofunctionalisation, cell rolling, phenotype modifications.

INTRODUCTION
Many available processes to biofunctionalise surfaces either require expensive and time-consuming protocols or are incompatible with the fabrication of microfluidic systems[1].
Our method differs from the existing since it is applicable to an assembled system, uses few microliters of reagents, lasts only few hours and it is based on the use of microbeads. The microbeads have specific surface moieties to link the biomolecules used to couple cell receptors and offer the benefit of the multivalent interaction[2]. We functionalised the systems with antibodies able to form complexes with the MHC class I (MHC-I) molecules present on the cell membrane, involved in the immune surveillance[3].
Such systems are used to roll mice tumor cell lines (RMAs) and change their phenotype. These changes are evaluated by FACS analysis and natural killer (NK) cells-mediated citotoxicity assays . The NK are lymphocytes of the immune system able to kill target cells lacking MHC-I expression[3].

EXPERIMENT
The microfluidic chambers (Figure 1left) were fabricated in PDMS and bond by plasma radicalization to a glass substrate.
Figure 2 shows the protocols of functionalisation. Just after the plasma bonding (Figure 2-1), 30% APTMS in Methanol is injected in the chamber and incubated for one hour at room temperature. The system is then washed by injecting DI water for 1 minute, dried by flushing N^2 for 3 minutes, and baked in the oven for 2 hours at 120°C (Figure 2-2); The biotin (2mg/ml), EDC (10mg/ml) and NHS (15mg/ml) in DI water are injected in the microfluidic chamber and incubated for 2 hours at 4°C (Figure 2-3). The microfluidic chamber is then washed by injecting PBS for 1 minute; Streptavidin beads, 2μm in diameter, 1.25% in buffer solution (PBS) are injected in the microfluidic chamber and incubated for 1 hour at 4°C (Figure 2-4). The chamber is then washed for 1 minute in PBS; The biotinylated antibody (0.1 mg/ml) in PBS is injected in the microfluidic chamber and incubated for 1 hour at 4°C (Figure 2-5); The microfluidic chamber is finally washed for 1 minute in PBS. Note that for the biofunctionalisation of the systems not integrating microbeads, the protocol used was the same but streptavidin (2mg/ml in PBS) was used instead of streptavidin beads.
To test the microfluidic system, RMAs were rolled in the device to recognise and strip MHC-I molecules. Each experiment was performed connecting in parallel two microfluidic systems to a syringe pump, the first chamber was opportunely biofunctionalised and the second chamber was used as control (without functionalisation). 350μl of sample (1500cells/μl in buffer solution, PBS) were loaded in each microfluidic chamber at a flow rate of 0.5ml/hr corresponding to a shear stress of 2dyne/cm^2.

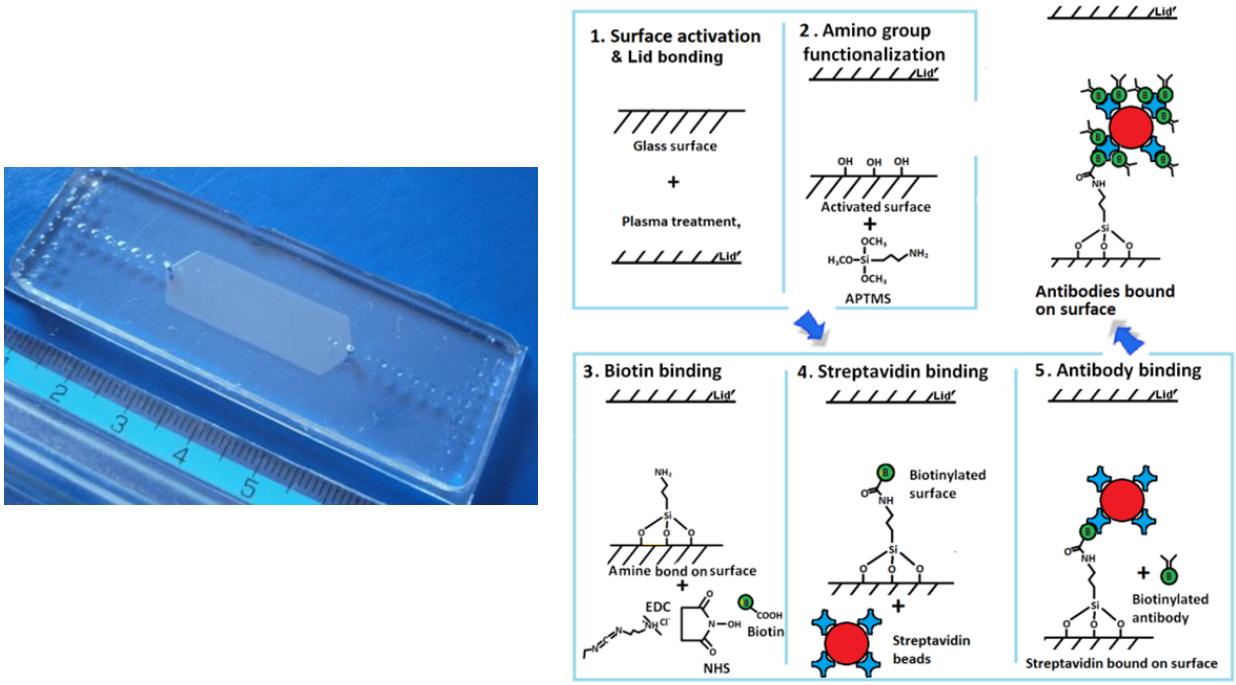

Figure 1: left: Isometric view of the PDMS microfluidic chamber (1cm wide, 2.5cm long and 35 μm high). It is plasma bonded on a glass slide (25mmx75mmx1mm);
right: Surface biofunctionalization process

RESULTS

Figure 3-left shows the surface coated by biofunctionalised beads which are distributed uniformly across the surface. Figure 3-right shows the selective binding between cells and biofunctionalised beads.

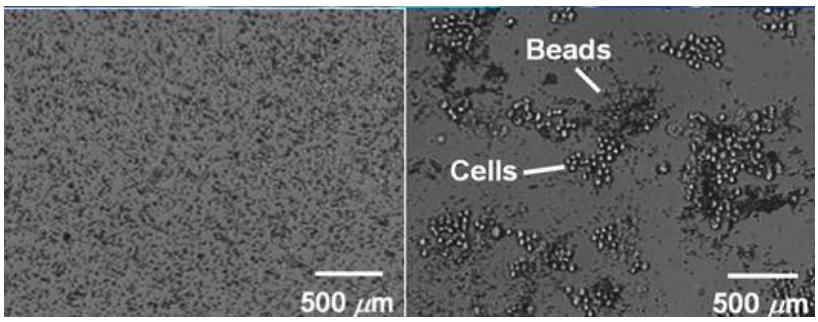

Figure 3: Left: Surface coated by streptavidin beads.
Right:. Cell rolling on a biofunctionalised glass substrate. Cells adhere where antibody-conjugated beads were present (an area where the distribution of beads was not homogenous was shown on purpose for demostrating the selectivity of the binding between cells and beads: the cells were bond on the beads only).

As result, we showed that cell rolling performed inside a microfluidic chamber functionalized with beads and the opportune antibody facilitate the removal of MHC class I molecules.
We showed that, after FACS analysis, the level of median fluorescent intensity (MFI) of the MHC-I molecules was 300 for cells treated in a not biofunctionalised surface. It decreased to 275 for cells treated in a flat biofunctionalised surface and to 250 for cells treated on a surface where biofunctionalised microbeads were immobilized (Figure 4). Figure 5 shows the cytotoxicity assays of cells recovered from biofunctionalised and a not biofunctionalised system for effectors(NK)/targets ratios (E:T) equal to 200/1, 100/1, 50/1, 25/1. The cells with reduced expression of MHC-I molecules showed after cytotoxicity tests an NK susceptibility 3.5 times higher than not treated cells.

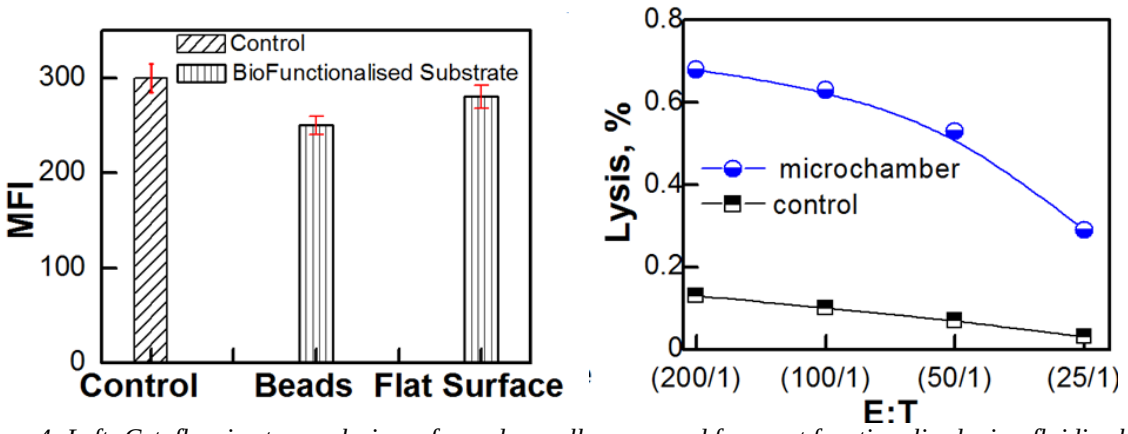

Figure 4: Left: Cytofluorimeter analysis performed on cells recovered from not functionalized microfluidic chambers (control), microfluidic chambers with flat bottom surfaces functionalised with MHC-I antibodies, and microfluidic chambers with immobilised beads on the bottom surface biofunctionalised with MCH-1 antibodies.
Right: Citotoxycity of cells according to different effector (NK) to target (RMAs) ratios of cell treated in the biofunctionalised and control microfluidic chamber

CONCLUSIONS

We demonstrated a flexible biofunctionalisation procedure for microfluidic devices applicable for different studies in cellomics. The developed microfluidic systems were biofuntionalized with antibodies to perform cell rolling of tumor cells, enhanced by selective biochemical binding of specific cell molecules. The data obtained showed the changes of the tumor cell phenotype by a reduction of MHC-I molecules on the cell membrane. We demonstrated that a reduction of MHC-I molecules induces an increased NK-mediated immune response against tumor cells.

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