A SIMPLE IN SITU MICROFLUIDIC PROCEDURE TO CREATE MULTIVALENT BIOFUNCTIONALIZED SURFACES.

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ABSTRACT

In this study we propose a fast, simple method to biofunctionalize microfluidic systems for cellomic investigations based on microfluidic protocols. The combination of biofunctionalized surfaces and microfluidics gives the opportunity to create devices that can better diagnose disease, or discover cell functionalities. The developed systems can be used for different application in the cellomic fields. In this work, they were tested to sort tumor cells and change tumor cell phenotypes. The results showed a high flexibility of the biofunctionalized protocols to create such systems in short time, with low reagent consumption and in a standard manner.

KEYWORDS: Biofunctionalization, cell handling.

INTRODUCTION

To reproduce in vitro the extracellular environment has been considered as an important issue to understand and study cell behavior. To this aim, microfluidics was adapted together with topology and bio-chemistry surface modifications [1]. Many processes either require expensive and time-consuming protocols or are incompatible with the fabrication of microfluidic systems [2]. Our method differs from the existing since it is applicable to an assembled system, uses few microliters of reagents and it is based on the use of microbeads. They have specific surface moieties to link the biomolecules and couple cell receptors. Microbeads serve as arm spacer and offer the benefit of the multivalent interaction [3]. Herein we propose two biofunctionalization protocols that can be used for cellomic applications, the first exploits streptavidin-biotin interaction to couple antibodies (biotinylated antibodies, common in the biotechnology market), the second can be used for biomolecules showing amino, hydroxyl groups but is extremely specific to form the β-isomer of carbohydrates.

Figure 1: Schematic representation of the biofunctionalization process.
EXPERIMENTAL

The microfluidic systems were fabricated, by plasma bonding of casted PDMS and native glass substrate, and biofunctionalised. Figure 1 schematizes the two biofunctionalization protocols. The glass substrates of both the systems were modified by amino groups (after the plasma bonding, 30% APTMS in Methanol is injected inside the chamber and incubated for 1 hour at 25°C, then washed with DI water and dried with N2). Following, epoxide (from poly(ethylene glycol) diglycidyl ether 10mM in NaHCO₃ pH 8.3, injected in the microfluidic chamber and incubated for 1 hr at 25°C) and biotin moieties (biotin (2mg/ml), EDC (10mg/ml) and NHS (15mg/ml) in DI water were injected in the microfluidic chamber and incubated for 2 hours at 4°C) were coupled directly to the amino groups. Epoxide was reduced by hydrazide beads, biotin coupled streptavidin beads. Hydrazide beads (3 µm, 10mM carbonate buffer, pH=8.3) were perfused into the systems and incubated at 25°C for 2 hr, whilst streptavidin beads (2 µm in diameter, 1.25% in PBS) were incubated at 4°C for 1 hr. Unbound beads were removed flowing PBS.

Figure 2. Left: Top view of 2 µm beads bound on the glass surface. Right: SEM shows the bead distribution, following the PBS washing.

Figure 2 shows the homogeneous distribution of beads linked to the bottom layer. Hydrazide moieties were conjugated by galactose specifically forming the β-isomer (crucial molecules in the interaction with tumor cells). Streptavidin coupled biotynylated antibodies (W6/32, 0.1 mg/ml in PBS, specific for the MHC class I molecules present on the cell membrane and involved in the immune surveillance).

RESULTS

Characterization of the biofunctionalization steps were performed demonstrating the presence and homogeneous distributions of the biomolecules (data not shown). To test the microfluidic systems, colon metastatic cell lines were incubated on carbohydrate beads and isolated (Figure 3a and 3b), whilst tumour cell lines (IM9) were rolled across the coupled antibodies to strip MHC class I molecules.

Figure 3a, shows the presence of tumour cell only on the biofunctionalised surface sorted from a mixed cell population.
Figure 4: Left: zoom-in of an area of a microfluidic chamber in which the distribution of biofunctionalized beads is not homogeneous. In this chamber tumor cells (IM9) were injected. It can be seen that the cells only bind on the beads on which a specific antibody (W6/32) is attached. This specific antibody binds on the MHC class I molecules present on the cell membrane. Rolling of cells in such microfluidic chamber allows stripping the specified molecules from the cell membrane. This finds applications in tumor therapies. Right: Confocal microscopy (seen in the FITC channel only) images of a tumor cell stained with fluorescent W6/32 antibodies which has been treated in the biofunctionalized microfluidic chamber (top) and one which has not been injected in the microfluidic chip (bottom). (the cells have a diameter of 10μm)

Figure 4 shows the interaction of the cells with the biofunctionalised surface (left) and confocal images of treated and non treated cells (right) stained with fluorescent antibodies specific for MHC class I molecules. It is evident that the treated cell shows less intense and more diffuse fluorescence, demonstrating a reduction of the MHC-I molecules

CONCLUSIONS
We showed an efficient and simple method to biofunctionalize microfluidic environments within five hours using few tens microliters of reagents and biomolecules. This method permits aiding the studies in cellomics. Two successful applications were shown. The first used the microfluidic system biofunctionalised with carbohydrates to sort metastatic cell lines. The second application used the microfluidic system biofunctionalised with antibodies to interact with tumor cells changing their phenotype. In conclusion, we demonstrated the flexibility of the biofunctionalization procedures to be used for different applications.

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REFERENCES

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