CELL ATTACHMENT AND PATTERNING ON POLYELECTROLYTES AND BACTERIAL PROTEINS

V. Saravia[1], S. Küpcü[2], D. Pum[2], U. B. Sleytr[2] and J. L. Toca-Herrera[3]

[1]Department of Chemical Engineering, URV, Av. Paisos Catalans 26, Tarragona, Spain
[2]Center for Nanobiotechnolgy, BOKU, Gregor-Mendel Str. 33, 1180 Vienna, Austria
[3]Biosurfaces Unit, CIC biomaGUNE, Paseo Miramón 182, 20009 San Sebastián, Spain

mariaveronica.saravia@urv.cat; jltocaherrera@cicbiomagune.es

Cells interactions with surfaces are relevant to many areas of science and technology, from biosensors to tissue engineering. According to the cell-surface interaction type, surfaces can be classified as follows: a) non-fouling, b) surfaces on which passive adhesion forces mediate primarily cell adhesion and c) surfaces on which cells have an active interaction [1]. In particular, for anchorage dependent cells, the attachment and spreading is mediated by proteins of the extracellular matrix (ECM) such as fibronectin, laminin and collagen [2]. The process of protein adsorption is affected by the properties of the surface (surface energy), the nature of the protein (hydrophobicity and charge), and the solution conditions (pH, ionic strength, etc) [3]. One of the most popular methods to avoid protein adsorption on a particular area is the use of poly(ethylen glycol) (PEG). Polymers that comprise carbohydrate units also passivate surfaces, but this type of material is less stable and less effective than PEG.
Bacterial cell surface layers (S-layers) are (glycol)proteins which molecules are able to self-assemble into 2-D crystalline structures on lipids, capsules and different type of substrates [4,5]. S-layer protein engineering technology, through the making of fusion proteins with defined biological functions, is increasing its importance in nanobiotechnology applications.
In this study, we investigated the attachment of a hepatoma cell line (HepG2) on surfaces with different chemistry. Polyelectrolyte coating with layer-by-layer technique was used to modified glass or polystyrene. The bacterial cell surface protein (SbpA) was isolated from *Bacillus sphaericus* CCM2177. Culture conditions, extraction and purification were carried out as described in [6]. SbpA recrystallization leading to the formation of S-layers, is carried out on polyelectrolyte modified substrates according to Toca-Herrera et al. (2005) [4]. The 2-D crystalline structure of the protein layer was resolved with atomic force microscopy.
The attachment of the cells to the substrates was studied by optical microscopy and the estimation of the viability of the cells using MTT assay (yellow tetrazolium (3-(4, 5 dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide). Figure 1 shows that cells were able to attach to the surface coated with anionic PSS (poly styrene sulfonate) and cationic PAH (poly allylamine hydrochloride), but their attachment was prevented by coating the surfaces with a polyelectrolyte grafted with PEG. Further studies on Spba recrystallization are being carried out showing that the protein layer prevents cell attachment. Besides, cell patterning will be attempt by combining cytophilic (PSS and PAH) and cytophobic surfaces (SbpA and PLL-g-PEG).

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Figure 1: Microscope pictures of adsorbed HepG2 cells on glass coated with polyelectrolytes. PAH, PSS and PLL-g-PEG were the polyelectrolyte layers in contact with the cells. After 48h of cultivation MTT was added. The blue spot shows that metabolically active cells are attached to the surface. PLL-g-PEG proved to avoid the cell attachment.