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The modulation of attachment, growth and morphology of cancerous prostate cells by polyelectrolyte nanofilms☆

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ABSTRACT

The behaviour of cancerous epithelial prostatic cells (PC3) growing on polyelectrolytes (PE) coatings was compared to the behaviour of immortalized normal prostatic cells (PNT-2). The cell behaviour was evaluated and quantified in terms of initial cell attachment, growth, metabolic activity, morphometry, adhesion, apoptosis and stress related gene expression. Both the anionic PSS (poly(sodium 4-styrenesulphonate))-terminated surface and cationic PAH (poly(allylamine hydrochloride))-terminated surfaces were not cytotoxic. The initial attachment of cells was better on the PAH-terminated surface compared to fibronectin. However, the proliferation rate of PC3 cells was reduced on the PAH-terminated surface and slightly increased on the PSS coatings. Only PAH prevented the clustering phenotype of PC3 and reduced the number of focal adhesion points as compared to fibronectin or PSS coatings. In contrast, none of the PE surfaces significantly affected the biological responses of PNT-2 cells. PAH-terminating films provide a tool to preferentially modulate the growth of some cancerous phenotypes, in this case as a micro-environment that reduces the growth of metastatic PC3 cells.

1. Introduction

Culturing cells ex vivo that differentiate and maintain in vivo characteristics holds great promise not only for the fundamental investigation of cell function but also in tissue engineering and regenerative medicine [1,2]. The ultimate commitment of a cell to differentiate, proliferate, migrate, undergo apoptosis or perform other specific functions is a well-coordinated response to its molecular interaction with the components of the extracellular matrix (ECM) and surrounding cells. Thus an ex vivo culture scaffold should mimic both the architecture and differentiated function of a given tissue while allowing experimental intervention. Progress in biomaterials design and engineering is converging to enable a new generation of instructive materials to emerge as candidates for regenerative medicine [3]. Von der Mark described the ultimate goal in the design of biomimetic materials for use in tissue engineering is to generate scaffolds with appropriate biomechanical and chemical properties to allow the adhesion, ingrowth, and survival of cells [4]. In addition to biochemical factors, mechanical interactions between cells and extracellular matrix also influence tissue architecture and, in the case of epithelial tissue, promote the polarity and mediate epithelial lumen formation [5]. More specifically tumour cells are known to sense, process and respond to mechanical forces in their environment as demonstrated by the effect of extracellular matrix stiffness on epithelial morphogenesis [6].

The development of new nanotechnologies such as photo- or electron-beam nanolithography, polymer demixing, nanoimprinting, compression moulding, or the generation of TiO_2 nanotubes of defined diameters (15–200 nm), has provided the

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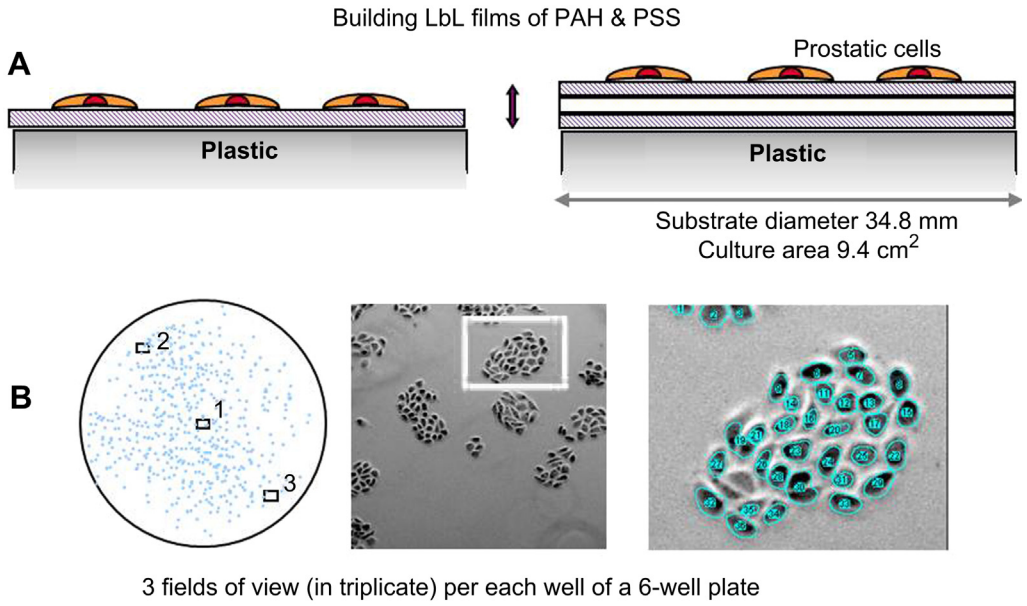

Fig. 1. Methodology. A. Schematic of the LbL-assembled PE-coated multiwell cell assays (1 layer on the left, 3 layers on the right). B. Cell counting method. 3 fields of view for cell counting were considered in each well, 2 fields in the opposite side of the well and one in the centre.

possibility to construct biomimetic surfaces with a defined nanopattern than can elicit tissue-specific cellular responses by stimulating integrin clustering [4]. However the manufacturing of biomimetic surfaces using such nanotechnologies requires large equipment and facilities. Thus, it is preferable to have a surface that self-assembles, self-organizes and can easily incorporate "cell-friendly" macromolecules [7]. Ideally, such a surface could also direct cell fate by means of material composition, bioactivity and mechanical properties (see Ref. [8] for review). Polyelectrolytes appear to provide a solution for such a surface since they allow the precise control of the substrate properties. Surfaces formed using polyelectrolytes could be of great advantage for the investigation of the behaviour of cells deposited on films. For example, in this report we utilized the properties of the LbL polyelectrolyte films to adjust the extracellular environment and analyse the responses of epithelial cancerous cells.

The construction of LbL (layer-by-layer) films using polyelectrolytes (PE) has the capability to provide a substrate that is non-cytotoxic [9–11]. Moreover, the surface properties of films are easily adjusted to be either positively or negatively charged with the appropriate choice of terminating layer. The thickness of the film can be adjusted by changing the number of LbL layers [9]. Such LbL nanoassembled structures are promising tools to modulate complex cellular processes, such as proliferation, adhesion, differentiation and have been extensively used for tissue engineering, tissue biomimetism and biomedical applications (see Refs. [8,12] for reviews). LbL polyelectrolyte platforms have also been previously used to discriminate healthy cells from cancerous breast cells [13].

In this study, we report our investigations of the influence of polyelectrolyte nanofilms on prostatic cells morphology, adhesion, growth and proliferation. We previously showed that the behaviour of a fibroblast-like pre-adipose cell line and a neuroepithelial-like cell line could be modulated by the final charge of PE [11]. Our current studies expand to a broader range of cell types the available information about the use of PE films as cancerous cell-culture substrates. Our results indicate that the phenotype of epithelial cancerous prostatic cells can be modulated by choosing the appropriate terminating polyelectrolyte to alter the surface charge of the LbL films. For example, a terminating layer of PAH, which gave the LbL film a positive surface charge, influenced the morphology and slowed the proliferation rate of cancerous prostatic cells. These studies have provided significant information on the ability of a self-assembled polyelectrolyte nanofilm to modulate the growth characteristics of prostatic cancerous cells.

2. Materials and methods

2.1. Preparation of LbL (layer-by-layer) PE (polyelectrolytes) and control films

Multi-layered polyelectrolyte films on multi-well plates were constructed by alternate coating of PSS (poly (sodium 4-styrenesulphonate; Sigma 243051)) and PAH (poly (allylamine hydrochloride; Sigma 283223)) following the LbL (Layer-by-Layer) process we have previously described for cell culture experiments [11]. The methodology is summarized in Fig. 1A. The substrate was alternately exposed to a solution of either PSS or PAH for 20 min on a rocking platform. Both the PSS solution and the PAH solution were made at a concentration of 1 mg/ml with 0.5 M NaCl. The films were constructed by commencing with either PSS or PAH, then alternating with PSS or PAH to construct the multilayered film and then terminating the film with either PSS or PAH as indicated in the presentation of the results. Each layer was approximately 2 nm thick [11]. Between the changes of polyelectrolyte solution, the 6-well plates were washed thoroughly with MilliQ-grade water (18 MΩ cm). In this study, films with 1, 2 or 3 alternated layers were utilized. Therefore, 6 different coatings were studied, corresponding to 4 different terminal layers which were either PSS (single layer), PAH (single layer), PAH/PSSter (multilayer terminated by PSS) or PSS/PAHter (multilayer terminated by PAH). We used untreated substrate (CT) (standard BD Falcon™ tissue culture-treated polystyrene plates, BD Biosciences, Ref. 353046) and fibronectin (FIBRO) coated substrates (Sigma F1141-5 mg) as controls. The film of fibronectin, at a concentration of 10 μg/ml, dissolved in phosphate buffered saline (PBS supplemented with Ca^{2+} and Mg^{2+} = PBS+) was coated on multi-well plates by incubation of 30 min at RT followed by a wash with PBS + for 10 min. All the coated substrates were stored at 4 °C before use.

2.2. Cell lines and culture on PE films

PC3 cells (prostate carcinoma) were obtained from ATCC (Ref. CRL-1435). The PC3 cells were initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old male Caucasian. PC3 cells were routinely cultured in RPMi Glutamax culture media (Invitrogen, Ref. 61870-010) supplemented with 10% foetal calf serum (PAA, Ref. A15-101) and 1% penicillin/streptomycin (Invitrogen, Ref. 15140-122). PNT-2 cells (normal prostate epithelia) were obtained from ECACC (N°95012613) and were routinely cultured in RPMi Glutamax culture media supplemented with 10% foetal calf serum and 1% penicillin/streptomycin. In order to minimize the protein adsorption on PE and thus to really take into account the effects of PE on cell behaviour, the rate of SVF in culture medium was drastically