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Protection of mammalian cell used in biosensors by coating with a polyelectrolyte shell

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Abstract

In order to detect xenoestrogens which induce perturbations of mammalian cells, design of biosensor using a mammalian cell line enable to detect these compounds is necessary. MELN cell line is suitable to detect estrogen activity, since they are stably transfect with an estrogen regulated luciferase gene. To realize this biosensor, it appeared necessary to add a protection to the mamalian cell, which is devoided, of the wall protecting yeasts or plant cells. With this aim in view, MELN cells have been isolated with a polyelectrolyte shell using the layer-by-layer technique. Among several polyelectrolyte-couples, the best cell survival (>80%) was obtained by alternating the polycation poly-diallyldimethyl ammonium chloride layer and the negatively charged poly-styrene sulfonate. We observed that the composition of the buffer used for layer-deposition was crucial to preserving cell viability, e.g. potassium ions were preferred to sodium ions during the coating. Furthermore, viability was increased when cells were allowed to recover for 2 h between each bilayer deposition. The use of engineered mammalian cells that synthesize luciferase as a response to exposure to estradiol, demonstrated that coating not only permits cell survival, but also allows essential metabolic functions, such as RNA and protein synthesis to take place. Capsule formation allows free diffusion of small molecules, while it prevents internalization in the cells of proteins larger than 60 kDa.

Keywords: Estrogen; Polyelectrolytes; Biosensor; Chemiluminescence; Cell coating

1. Introduction

Environmental estrogens, such as endogenous plant compounds or xenoestrogens (pesticides, estrogenic drugs ...) have been reported by several epidemiological studies to be in association with the incidence of some hormone-dependant diseases (Roy et al., 1998; Andersen et al., 2002; Lei et al., 2002). For example, exposure to environmental estrogens has been associated with an increase in incidence of testicular and breast cancer, and compromised reproductive capacity (Byford et al., 2002; Diel et al., 2002). To realize a biosensor able to detect molecules displaying a hormone or anti-hormone-like activity on mammals, we used a mammalian cell line: MELN derived from MCF-7 human breast cancer cells stably transfected with an estrogen regulated luciferase gene (Balaguer et al., 1999, 2001). Realization of in vitro or in vivo biosensors using

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mammalian cells require to isolate them from environment. One way to isolate mammalian cells is to microencapsulate them.

Obtention of microencapsulated mammalian cells is not a recent goal: it has been first proposed to immunoprotect cells grafted in a live organism, and thus prevent graft rejection by coating cells using ultra thin polymer membranes (Chang, 1964). This approach was further developed for therapeutic treatments, e.g. diabetes (Lim and Sun, 1980), haemophilia (Liu et al., 1993) and liver failure (Chang, 1992). In the field of biotechnology, other applications are related to large-scale production of cell-derived molecules (Scheirer et al., 1983), in vitro culture of cells dependent on close cell–cell contact (Loty et al., 1998) or cellular biosensors (Goguen and Kedersha, 1993; Okada et al., 1996). All these applications require that the encapsulation procedure does not alter cellular functions. The capsule should be semi-permeable, allowing the free bi-directional diffusion of molecules, oxygen, nutrients and therapeutic molecules, should be able to across the barrier, whereas high molecular weight molecules, such antibodies and immune cells (like lymphocytes) should be excluded (Dove, 2002; Orive et al., 2002, 2003). Further requirements are the biocompatibility of the capsule, its mechanical and chemical stability, its functional performance and long-term survival of the encapsulated cells (Uludag et al., 2000).

A semi-permeable membrane can be obtained by deposition of several layers of oppositely charged polyelectrolytes to generate capsules; this method is called the layer-by-layer (LbL) technique (Decher, 1997; Caruso, 2000). Each individual layer has a thickness between 2 and 5 nm and a permeability cut-off of 10–30 kDa at physiologic pH, varying according to the ionic strength and the nature of the polyelectrolytes (Ladam et al., 2000; Gao et al., 2001, 2003; Sukhorukov et al., 2001). This low exclusion size should allow the differential bi-directional diffusion of molecules and one application of this LbL technique is the encapsulation of cells.

Cell encapsulation has been achieved by prior embedding into an alginate bead. To strengthen these alginate beads and to control their porosity (preventing the passage of large molecules), they were covered with either one poly-L-lysine layer (De vos et al., 2002) or with several layers of oppositely charged polyelectrolytes (Schneider et al., 2001). This should have improved immuno-protection when the beads were grafted into the animal. This technique was also successfully used to coat glutaraldehyde-fixed, dead erythrocytes (Donath et al., 2002), living yeast (Diaspro et al., 2002; Krol et al., 2005) and mammalian tissues (Thierry et al., 2003). Fragile cells, such as living mammalian cells cultured in vitro, however, have never been encapsulated without previous embedment.

The aim of our work was to design a procedure that resulted in the coating of mammalian cells in a polyelectrolytes shell using the LbL technique. We used the cell line MELN, engineered to synthesize the enzyme luciferase, as a response to estradiol exposure. Preliminary experiments of cell coating using the LbL technique were unsuccessful due to the fragility of these mammalian cells. To solve this problem, we tested different cell coating conditions and controlled for cell viability. Once the coating conditions were optimal in terms of cell survival and capsule formation, we analyzed the influence of the capsule on estradiol induction of luciferase, and we verified the wall permeability, permeable to low molecular weight molecules, such as steroid hormones, and impermeable to high molecular weight molecules, such as proteins.

2. Experimental

2.1. Materials

We chose adherent cells to facilitate the elimination of polyelectrolytes between each layer deposition. Cell line MELN was derived from the MCF-7 breast cancer cells that express estrogen receptor α (ER α). MCF-7 cells were stably transfected with a construct expressing the luciferase gene under the control of an estrogen-regulated promoter (Balaguer et al., 1999, 2001).

We used the following polyelectrolytes from Sigma–Aldrich. Polycations: poly(ethylene imine) (PEI, MW: 25,000 Da, 1 mg/mL), polyphosphoric acid (PPP, MW: 340 Da, 1 mg/mL), poly(allylamine hydrochloride) (PAH, MW: 70,000 Da, 1 mg/mL), protamine sulfate (PS, MW: 4000 Da, 1 mg/mL), poly-L-lysine (PLL, MW: 50,000 Da, 0.1 mg/mL) and poly(diallyldimethyl ammonium) chloride (PDADMAC, MW: 150,000 Da, 2 mg/mL). For the polyanion, we used poly(sodium 4-styrene sulfonate) (PSS, MW:70000 Da, 1 mg/mL). PDADMAC has been labeled using tetramethyl rhodamine isothiocyanate (TRITC). Polyelectrolytes were dissolved in different solutions: HEPES/NaCl (25 mM HEPES, 145 mM NaCl, pH 7.4), Ringer/KCl (10 mM HEPES, 147 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM glucose, 2 mM sodium bicarbonate and pH 7.4). Cellular viability was analyzed with two DNA dies: SYTO-16 (Molecular Probe) and propidium iodide (Sigma). All binding studies based on surface plasmon resonance (SPR) phenomenon were performed on a four-channel BIACORE 3000 optical biosensor instrument (BIAcore AB, Uppsala, Sweden), and the experiments were performed on the carboxylated surface of a sensorchip C1 (BIAcore SA sensorchip).

For internalization experiments, the HeLa cell line was used, because internalization of lectins and transferrin in this cell line is well documented (Francis et al., 2003). Lectin from *Xerocomus chrysenteron* (XCL) and primary antibody directed against lectin, were produced as previously described (Francis et al., 2003). Fluorescein-conjugated transferrin from human serum and secondary antibody (FITC-labeled anti-rabbit) were purchased from Molecular Probes.