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Linker-free covalent attachment of the extracellular matrix protein tropoelastin to a polymer surface for directed cell spreading

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Abstract

Polymers are used for the fabrication of many prosthetic implants. It is desirable for these polymers to promote biological function by promoting the adhesion, differentiation and viability of cells. Here we have used plasma immersion ion implantation (PIII) treatment of polystyrene to modify the polymer surface, and so modulate the binding of the extracellular matrix protein tropoelastin. PIII treated, but not untreated polystyrene, bound tropoelastin in a sodium dodecyl sulfate (SDS)-resistant manner, consistent with previous enzyme-binding data that demonstrated the capability of these surfaces to covalently attach proteins without employing chemical linking molecules. Furthermore sulfo-NHS acetate (SNA) blocking of tropoelastin lysine side chains eliminated the SDS-resistant binding of tropoelastin to PIII-treated polystyrene. This implies tropoelastin is covalently attached to the PIII-treated surface via its lysine side chains. Cell spreading was only observed on tropoelastin coated, PIII-treated polystyrene surfaces, indicating that tropoelastin was more biologically active on the PIII-treated surface compared to the untreated surface. A contact mask was used to pattern the PIII treatment. Following tropoelastin attachment, cells spread preferentially on the PIII-treated sections of the polystyrene surface. This demonstrates that PIII treatment of polystyrene improves the polymer's tropoelastin binding properties, with advantages for tissue engineering and prosthetic design.

Keywords: Plasma immersion ion implantation; Tropoelastin; Covalent linkage; Polystyrene; Cell spreading

1. Introduction

Polymers are used for the fabrication of many prosthetic implants such as vascular grafts, heart valves, bone implants and joints, parenchyma and in reconstructive surgery [1,2]. Some biopolymers used for tissue engineering are required to be inert in order to prevent biological activity, e.g. for joint prosthesis, intraocular lenses and blood-contacting devices, whereas it is desirable for others, such as for bone and skin implants, to promote biological function by promoting the adhesion, differentiation and viability of cells [2]. Extracellular matrix (ECM) proteins mediate cell attachment signals that can be utilized in the generation of novel hybrid biomaterials [3].

Polymer surfaces are typically either too hydrophobic or negatively charged to adhere cells directly, and therefore many polymers are modified to enhance cell binding. Physical methods of surface modification are appealing for manufacture, and include electrostatic treatment [4], carbon deposition [5], UV/gamma irradiation [6,7], plasma discharge [8] and ion implantation [1,9–11]. These surfaces bind to cells via receptor and non-receptor mechanisms, and so may not elicit the cell signals that cells would receive from ECM proteins [2]. Cellular interactions with ECM are vital for cell survival and tissue maintenance and are involved in many biological functions such as cell migration and proliferation, tissue organisation, wound repair, development and host immune responses [12]. A lack of these signals often results in cellular dedifferentiation and

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apoptosis. The ability to covalently attach biologically active ECM proteins to the surface enables biological control of cellular activity [13].

Simple physisorption of ECM proteins, as often used to coat surfaces with molecules such as fibronectin and collagen, is non-specific, relying on multiple weak hydrophobic, van der Waals or permanent dipole interactions. Binding through these interactions results in variable extents of attachment, persistence and conformational stability [14,13]. Covalent interactions offer the opportunity to present the attached ECM proteins in a conformationally more relevant state. Furthermore, strongly fixing proteins to surfaces enhances the resorption resistance of the protein. Many methods employed for covalent protein linkage require chemical linker molecules such as disulfides, silanization, epoxides or glutaraldehyde [15–17], which can result in chemical modification of active residues, may have adverse biological effects and involve more complex surface attachment processes.

In order to overcome problems associated with the use of whole proteins, short synthetic peptides are often used to improve cell adhesion. Examples include the integrin-binding peptide RGD [18,19] and the proteoglycan-based peptide KRSR [20], but these peptides do not have the full functionality and receptor specificity of the native ECM protein [14]. For example, many ECM proteins contain synergy sites [21] which are required for integrin specificity, and/or multiple receptor binding sites which are required for cellular signalling [22]. However a major barrier to the use of ECM molecules is material-induced random folding which may sterically hinder binding [23].

Unlike previous studies which explored the direct cell adhesive activity of plasma ion immersion implantation (PIII)-treated surfaces, in this paper, we explore the use of PIII to provide a patternable platform for enhanced cell spreading through the attachment of an ECM protein, tropoelastin. We have chosen the PIII methodology as it has been previously shown to allow linker-free covalent attachment of bioactive molecules to polymer surfaces [24,25]. Polystyrene was chosen because it is the most common material for culturing cells, and is the most widely applied surface for studying cell–material interactions. We have chosen the ECM protein tropoelastin to direct cell interactions because it interacts with a range of biologically relevant cell surface receptors [26–28].

2. Materials and methods

2.1. Materials

Biaxially oriented 0.1 mm thick polystyrene sheets were obtained from Goodfellows. Recombinant tropoelastin was produced in-house as described in Ref. [28]. The mouse anti-human elastin antibody, BA-4, and the goat anti-mouse IgG-HRP conjugated secondary antibodies were purchased from Sigma. Human dermal fibroblasts (HDFs) were sourced from the Coriell Research Institute (Camden, NJ, USA). Unless stated otherwise all other reagents were purchased from Sigma.

2.2. PIII treatment

Polystyrene sheets were cut into 0.8 cm × 8 cm strips and wiped with 100% ethanol. Samples were mounted onto a substrate holder, covered by an electrically connected mesh and immersed in an inductively coupled radiofrequency (RF) plasma. Pulses of high voltage bias were applied to the substrate holder as in PIII. The RF power was 100 W. The working gas pressure was 2 mtorr of high-purity nitrogen with a flow rate of 72 standard cubic centimeters (sccm). The samples were PIII treated by applying 20 kV pulses lasting for 20 μs with a repetition rate of 50 Hz to the sample holder for 800 s. The sample holder was earthed between the pulses. Further details of the treatment process and its application to the surface attachment of bioactive enzymes can be found in Refs. [24,30,31]. Untreated controls did not undergo treatment in the plasma chamber.

A contact mask in the form of 3 mm wide ADH Kapton blocking adhesive tape (Associated Gaskets, Australia) was used to limit PIII treatment to specific regions of the polystyrene. After PIII treatment the tape was removed.

2.3. SNA blocking

Tropoelastin was blocked with sulfo-NHS acetate (SNA) as previously described [29]. Briefly, tropoelastin was solubilized in 100 mM $NaHCO_3$, pH 8.5 to 1 mg ml^{-1} and a 25-fold molar excess of SNA (Pierce) was added and incubated at room temperature for 1 h. After incubation the excess SNA was removed by dialysis against four 1 l volumes of phosphate-buffered saline (PBS) at 4 °C. A control without SNA was included alongside the SNA-treated sample. Following dialysis the absorbance at 280 nm was measured and used to determine the tropoelastin concentration.

2.4. ELISA

Strips of untreated and PIII-treated polystyrene were cut into 0.8 cm × 0.8 cm squares and placed into the wells of a 24-well plate (Greiner). SNA-treated or untreated tropoelastin was diluted to the appropriate concentration in PBS and 0.75 ml added per well and incubated at 4 °C for 16 h. Unbound tropoelastin was removed by aspiration, and the samples were washed with 3 × 1 ml aliquots of PBS. The samples were washed by transferring to 1.5 ml 0.05% Tween (v/v) in PBS for 10 min at room temperature, 1.5 ml of 1 M NaOH for 10 min at room temperature, or to 1.5 ml of 5% SDS (w/v) in PBS and incubated at 90 °C for 10 min. Non-treated samples were washed in 3 × 1 ml PBS. The samples were returned to the 24-well plate and washed with 3 × 1 ml PBS. Non-specific binding to the polystyrene was blocked with 3% (w/v) bovine serum albumin (BSA) in