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Controlling multi-function of biomaterials interfaces based on multiple and competing adsorption of functional proteins

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ABSTRACT

Multifunctional biomaterial surfaces can be created by controlling the competing adsorption of multiple proteins. To demonstrate this concept, bone morphogenetic protein 2 (BMP-2) and fibronectin were adsorbed to the hydrophobic surface of polychloro-*para*-xylylene. The resulting adsorption properties on the surface depended on the dimensional and steric characteristics of the selected protein molecule, the degree of denaturation of the adsorbed proteins, the associated adsorption of interphase water molecules within the protein layers, and the aggregation of proteins in a planar direction with respect to the adsorbent surface. Additionally, a defined surface composition was formed by the competing adsorption of multiple proteins, and this surface composition was directly linked to the composition of the protein mixture in the solution phase. Although the mechanism of this complex competing adsorption process is not fully understood, the adsorbed proteins were irreversibly adsorbed and were unaffected by the further adsorption of homologous or heterologous proteins. Moreover, synergistic biological activities, including cell osteogenesis and proliferation independently and specifically induced by BMP-2 or fibronectin, were observed on the modified surface, and these biological activities were positively correlated with the surface composition of the multiple adsorbed proteins. These results provide insights and important design parameters for prospective biomaterials and biointerfaces for (multi)functional modifications. The ability to control protein/interface properties will be beneficial for the processing of biomaterials for clinical applications and industrial products.

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1. Introduction

Although the adsorption of molecules on material surfaces is a basic and intuitively understood phenomenon, the mechanisms governing protein adsorption are complex [1]. Many studies have been performed [2,3] to elucidate protein-surface interactions and provide guidance in designing new biomaterials. Consensus on the detailed mechanism of protein adsorption has not yet been achieved [2], and engineering approaches for manipulating protein molecules on material interfaces has not been established [4,5]. Current technologies may provide solutions to some of these problems and enable the creation of a biological environment that prevents further adsorption of non-specific proteins, widely known as non-fouling surfaces [6]. Consideration of protein adsorption tends to be limited to prevention in the design of new biomaterials, and improving biocompatibility appears to be the only useful application of protein adsorption [7,8].

The present study employs a novel focus on utilizing functional proteins and exploiting the protein-surface interface to achieve a multifunctional platform for guided biological activities via the controlled adsorption of multiple functional proteins. The concentration of irreversibly bound protein molecules (compared to the fraction of reversibly desorbed proteins during the rinsing process) [2] was characterized by a combination of QCM and protein assays. A relatively low concentration of adsorbed protein was observed, in contrast to reported concentrations obtained by covalent attachment approaches [9]. However, effective and sustained biological activities were confirmed based on the osteogenesis activity of the adsorbed bone morphogenetic protein 2 (BMP-2) and induction of proliferation by fibronectin. As a proof-of-concept, controlled display of these two activities on one material surface was accomplished by allowing multiple and competing adsorptions of BMP-2 and fibronectin; the resulting biological func-

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tions of osteogenesis and guided differentiation were induced not only with specificity but temporally. The resulting adsorption properties on the surface involved dimensional and steric effects of the selected protein molecules, the degree of denaturation of the adsorbed proteins, the associated adsorption of interphase water molecules within the protein layers, and the aggregation of proteins in the planar direction with respect to the adsorbent surface. A defined surface composition was formed by the competing adsorption of multiple proteins, and this surface composition was directly linked to the composition of the protein mixture in the solution phase. These properties were directly and indirectly linked to the alteration of the characteristics of the adsorbent surface by adsorption, and the parameters were manipulated and modified. Although the complicated mechanism remains to be clarified [10], the competing adsorption of multiple functional proteins can be accomplished readily and intuitively, in contrast to complicated immobilization techniques involving covalent grafting or specific binding approaches [11–13], which require substantial knowledge of both protein chemistry and materials chemistry. In addition, conferring advanced and biomimetic functions to biomaterials and the biointerfaces will enable more sophisticated and defined microenvironments for cell/surface interactions, which have been conventionally limited to small molecules, short peptide sequences, and synthetic molecules and analyses of only straightforward and superficial properties, such as hydrophobic/hydrophilic properties and cell adhesion properties. The present study provides insights for the design of prospective biomaterials and related clinical and industrial products.

2. Experimental section

2.1. Surface modification of the PPX-C coating

The PPX-C coating was prepared by a custom-built chemical vapor deposition (CVD) system comprising a sublimation zone, a pyrolysis furnace, and a deposition chamber. During the CVD polymerization process, dichloro-[2,2]-paracyclophane was first vaporized at approximately 150 °C and then transported to the pyrolysis furnace, where the dimer was pyrolyzed into monomer radicals at 670 °C. The radicals then entered the deposition chamber and polymerized on a rotating holder maintained at 15 °C to form a uniform PPX-C coating. To inhibit residual deposition, the temperature of the chamber wall was maintained at 90 °C. A stream of argon at a flow rate of 25 sccm was used as a carrier gas. Throughout the CVD polymerization, the operation pressure was regulated at 75 mTorr, and the deposition rate was maintained at approximately 0.5 Å/s, which was monitored by in situ QCM analysis (STM-100/MF, Sycon Instruments, USA).

2.2. Protein adsorption

Recombinant human BMP-2 (R&D Systems, USA) and fibronectin from human plasma (R&D Systems, USA) were obtained commercially. BMP-2 was reconstituted as a stock solution at a concentration of 100 μg/mL in sterile 4 mM HCl and stored at −20 °C. Fibronectin was reconstituted as a stock solution at a concentration of 100 μg/mL in phosphate-buffered saline (PBS, pH 7.4, Sigma Aldrich, USA) and stored at 4 °C. Adsorption was performed by incubating the protein solutions on PPX-C-coated substrates at 4 °C for 10 min. After protein incubation, a rinsing protocol of three rinses with PBS (pH 7.4, containing Tween 20, Sigma-Aldrich, USA), one rinse with PBS (without Tween 20), and one rinse with deionized water was employed to remove the loosely adsorbed proteins. The mixed BMP-2/fibronectin protein solutions were prepared by varying the mixture ratio at 1:0, 10:1, 1:1, 1:10, and 0:1, based on mass concentration.

2.3. Characterizations

Surface plasmon resonance (SPR) analysis was performed using a Biacore X-100 system (GE Healthcare, Sweden) equipped with a monochromatic and plane-polarized excitation light source. Standard SPR gold substrates (GE Healthcare, Sweden) were coated with PPX-C via the aforementioned CVD polymerization process, and a quartz prism was used to direct the light to the sample surface for analysis. A surface modified by PEG (containing thiol-terminal, MW 5000, Sigma-Aldrich, USA), which was immobilized on the maleimide-parylene-coated SPR substrate following a previously reported procedure [14], was used in parallel as a control surface. An automated handling device was used to inject buffer and protein solutions. The SPR experiments were performed at a constant flow rate of 5 μL/min at 25 °C, and each sample was measured in triplicate. QCM analysis was performed with an ADS-QCM instrument (ANT Technologies, Taiwan) equipped with a flow injection analysis (FIA) device and a continuous frequency variation recording device. The flow rate was controlled by a peristaltic pump connected to the FIA device. The sensing element of the instrument was an AT-cut piezoelectric quartz disc with a 9-MHz resonant frequency and a 0.1-cm^2 total sensing area. The quartz discs were also coated with PPX-C via the CVD polymerization process and used for protein adsorption. Protein solutions with a mass concentration of 100 μg/mL in PBS were injected through the FIA device to the analysis chamber, and the time-dependent change in frequency was continuously monitored. To examine antibodies by QCM, QCM crystals were prepared by PPX-C coating as the first step, followed by the adsorption of fibronectin or BMP-2, and the resulting crystal samples were then allowed to undergo dynamic analysis by introducing antibodies, including human fibronectin antibody (50 μg/mL in PBS, R&D Systems, USA) and human BMP-2 antibody (50 μg/mL in PBS, R&D Systems, USA), to the sample surface. Bovine serum albumin (BSA, 50 μg/mL in PBS, Sigma Aldrich, USA) and fibroblast growth factor 2 (FGF-2, 50 μg/mL, R&D Systems, USA) was also used in parallel as control proteins. All experiments were performed at a constant flow rate of 5 μL/min at 25 °C, and each sample was measured in triplicate. The protein surface characterizations by AFM were conducted using a MultiMode 8 instrument (Bruker, USA) capable of peak force tapping mode in liquids. The data were acquired using silicon nitride tips (Bruker, USA) with a tip radius of 12 nm and a spring constant of 0.04 N/m. Modulus images were recorded using a loading force of 0.3–0.5 nN and were collected on a 500×500 nm matrix area. During AFM analysis, the samples were placed in the analysis chamber in PBS (pH 7.4) at 20 °C. Image analysis was performed using NanoScopeAnalysis software.

2.4. Cell proliferation

The surfaces of cell culture plates (12 well, Corning, USA) were modified using the aforementioned PPX-C coating and protein adsorption procedures before use in cell culture. pADSCs isolated from subcutaneous adipose tissues [15] were seeded at a density of 1×10^4 cells/cm^2 and cultured on the modified surfaces of cell culture plates. Cell culture was performed in basal proliferation medium comprising Dulbecco's modified Eagle's medium with nutrient mixture F-12 (DMEM/F12; Gibco Laboratories, USA) containing 10% fetal bovine serum (FBS; Biological Industries, Israel), 100 kU/L penicillin (Sigma Aldrich, USA), 100 mg/L streptomycin (Sigma Aldrich, USA) and 1.5 mg/L amphotericin B (Biological Industries, Israel) at 5% CO_2, 37 °C and 100% humidity. Cell culture was conducted for 24 h and 72 h. The resulting samples were

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