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Mediating Mesenchymal Stem Cells Responses and Osteopontin Adsorption via Oligo(ethylene glycol)-amino Mixed Self-assembled Monolayers

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Oligo(ethylene glycol) (-OEG) and amino ($-NH_2$) mixed self-assembled monolayers (SAMs) were employed as model substrates to investigate the effect of charge density on the fate of mesenchymal stem cells (MSCs) and osteopontin (OPN) adsorption. We found that all surfaces presenting $-NH_2$ groups favored cell responses regardless of the surface charge. Meanwhile, OPN adsorption could remain stable on the mixed SAMs over a certain range of charge densities. Our work provides some insights into cell responses and protein adsorption to surface charge.

1. Introduction

Biomaterial surfaces with the ability to elicit a specific cellular response are beneficial to the design of functional biomedical materials and devices[1]. Surface properties can greatly influence proteins adsorption and subsequent activities of cells[2]. Self-assembled monolayers (SAMs) are a powerful tool to explore surface-related interactions by defining the surface chemistry. In particular, mixed SAMs are useful for fabricating gradients of interfacial composition, which can simplify material surface properties such as wettability and charge[3]. Although considerable research has shown that oligo(ethylene glycol) (-OEG) and amino ($-NH_2$) functionalities greatly affected cell adhesion and protein adsorption[4], few studies are dedicated to performance of -OEG/$-NH_2$ mixed SAMs on the stem cell response and protein adsorption. Here, -OEG/$-NH_2$ mixed SAMs were prepared to present close hydrophilicity but different charge densities. We investigated how -OEG/$-NH_2$ mixed SAMs mediated the fate of mesenchymal stem cells (MSCs) and osteopontin (OPN) adsorption. Specifically, OPN adsorption and subsequent cell adhesion on these SAMs over a range of charge densities were studied. Our work may provide a useful guidance on the design of biomaterial surfaces.

2. Experimental

After being cleaned by a nitrogen plasma treatment, gold slides were immediately immersed in mixtures including (11-mercaptoundecyl)hexa(ethylene glycol) ($HS(CH_2)_{11}(OCH_2CH_2)_6OH$, Sigma, USA) and 11-amino-1-undecanethiol, hydrochloride ($HS(CH_2)_{11}NH_2HCl$, Dojindo, Japan) with varying volume ratios (-OEG/$-NH_2$ = 10/0, 7/3, 5/5, 3/7, 0/10) at 1 mmol/L for overnight. The SAM-covered substrates were rinsed with ethanol, and then dried with a stream of nitrogen gas. The elemental compositions and chemical functional groups were analyzed by X-ray photoelectron spectroscopy (XPS, Axis-Ultradld, Kratos, UK) and Fourier transform infrared attenuated total internal reflection spectroscopy (FTIR-ATR, Vector 33, Bruker, Germany), respectively. The surface wettability and charge were investigated by a contact angle meter (OCA15, Data Physics, Germany) ($n = 6$) and an electrokinetic analyzer for solid surface analysis (SurPass, Anton Paar Austria) ($n = 4$), respectively.

Mouse bone marrow mesenchymal stem cells (mMSCs) (CRL-12424, ATCC, USA) were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS). Cells in early

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passages (≤6) were seeded on samples (10 mm ×10 mm) at a density of 1×10^4 cells cm^{-2} for proliferation and 5×10^4 cells cm^{-2} for adhesion and osteogenic differentiation. Cell morphology was observed by confocal laser scanning microscopy (CLSM, Leica TCS SP5, Germany) ($n = 2$). Cell proliferation was evaluated by a Cell Counting Kit-8 (CCK-8, Dojindo, Japan) assay ($n = 5$). The osteogenic differentiation of MSCs was evaluated by the RT-PCR reaction as reported ($n = 4$)[3].

Recombinant mouse osteopontin (OPN, R&D Systems, USA) was ^{125}I-radiolabelled using the iodogen method[5]. The amount of adsorbed OPN (ng/cm^2) was calculated from radioactivity using the following equation ($n = 5$).

$$\text{Protein} = \frac{\text{Count}}{A_{\text{solution}} S} \quad (1)$$

where count represents radioactivity of samples (cpm), A_{solution} (cpm/ng) and S (cm^2) are radioactivity of protein solution (10 μg mL^{-1}) and surface area (4 mm × 4 mm), respectively. The accessibility of cell-binding domains of adsorbed OPN was assessed by ELISA using osteopontin monoclona antibody (OPN mAb, R&D Systems, USA) ($n = 5$). After BSA blocking procedures, cells were seeded onto pre-adsorbed OPN surfaces and cultured in serum-free medium. Cell morphology was observed as described above ($n = 2$).

A statistical comparison was determined by analysis of variance (ANOVA) followed by a post-hoc test, and $p < 0.05$ was considered to be statistically significant.

3. Results and Discussion

As shown in Fig. 1(a), the S2p XPS spectra exhibited a doublet structure at approximately 162 eV, and there were no unbound thiol molecules (164 eV) and oxidized sulfur species (168 eV). This observation demonstrated that sulfur head groups had chemically adsorbed onto Au substrates[6]. In the FTIR-ATR spectra (Fig. 1(b)), absorption bands of -CH_2- appeared at 2919 and 2851 cm^{-1}, indicating that alkyl chains packed in crystalline fashion and further supported the

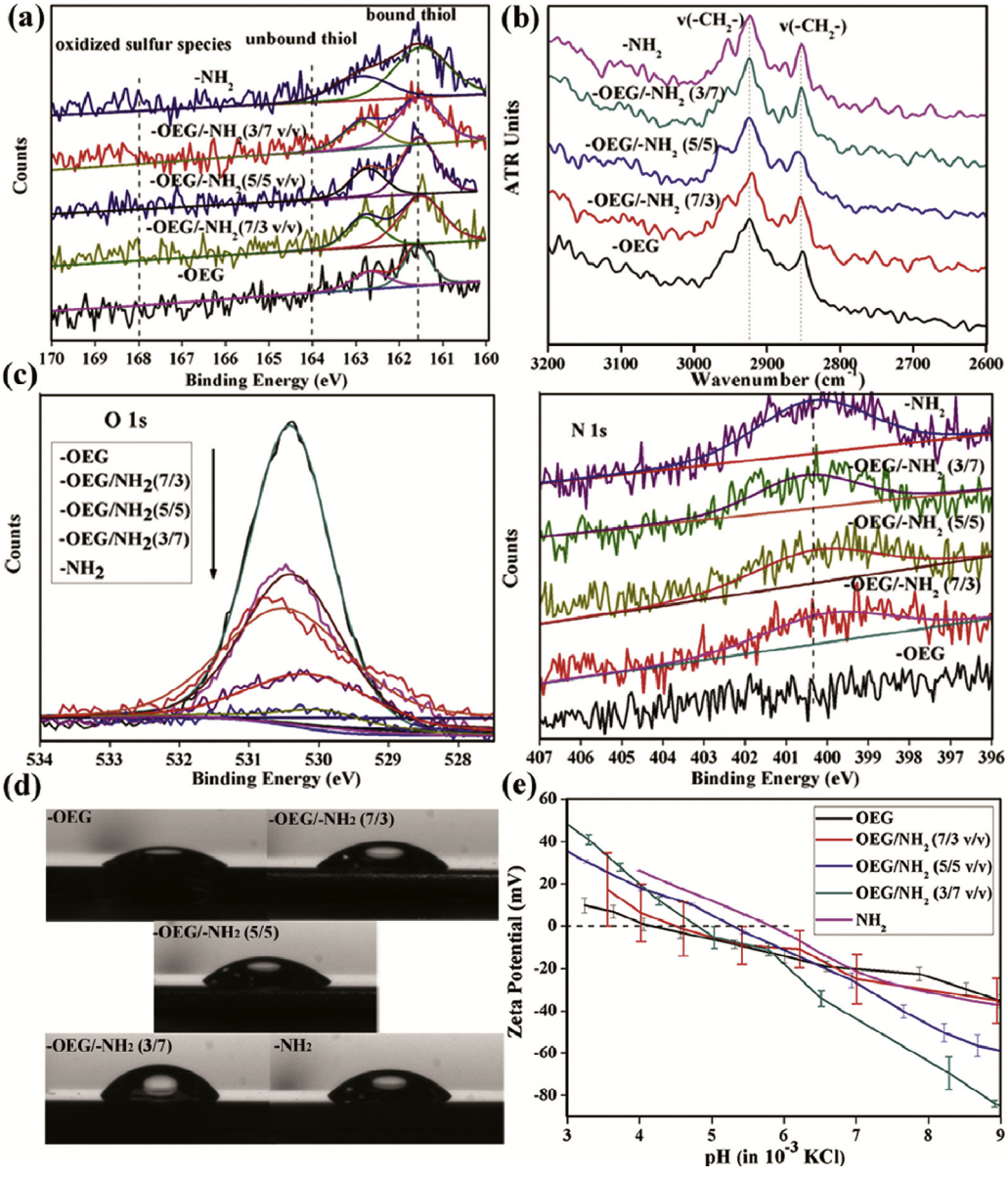

Fig. 1. Surface characterization of the -OEG/-NH_2 mixed SAMs: (a) S2p XPS spectra; (b) FTIR-ATR; (c) typical XPS spectra; (d) water contact angle, and (e) Zeta potential.