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Adsorption force of fibronectin controls transmission of cell traction force and subsequent stem cell fate

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ABSTRACT

The transmission of cell traction force (CTF) to underlying biomaterials is essential for adhered cells to measure and respond to their mechanical microenvironment. Given that the protein layer adsorbed on materials lies between the cells and materials, we hypothesize that the interfacial strength of protein-material interfaces (i.e., the adsorption force of proteins, F_{ad}) should have an important role in regulating the transmission of CTF. To test this hypothesis, rat mesenchymal stem cells (rMSCs) were cultured on poly(dimethyl siloxane) (PDMS) substrates with different F_{ad} of fibronectin (FN), and the transmission of CTF was observed by immunofluorescence staining of FN and deformation of PDMS. As revealed, FN on substrates with low F_{ad} is more liable to be desorbed by CTF, which prevents the transmission of CTF to substrates. In contrast, high F_{ad} facilitates the transmission of CTF from rMSCs to the FN layer and PDMS substrates so that rMSCs can perceive the mechanical properties of substrates. We further demonstrated that the divergent transmission of CTF on low and high F_{ad} substrates regulates the lineage specifications of rMSCs. Our study confirms the important role of F_{ad} in CTF transmission and provides a new perspective to gain insights into cell-material interactions and cell fates, which may help to guide the design of better biomaterials.

1. Introduction

Biomaterials act as artificial extracellular matrix (ECM) to provide adherent cells with physical support and living microenvironment. Appropriate cell-material interactions are the prerequisite for the success of biomaterials in tissue repair and regeneration, among which cell adhesion on biomaterials is the most important event [1,2]. Once adhered, cells will generate tensions through actin cytoskeletons and exert traction forces on the underlying materials through focal adhesions (FAs), fibrillar adhesions (FBs) and integrins [3–6]. By this way, cells sense and gauge the mechanical properties of materials to direct subsequent cell responses [7,8]. For example, cells can feel the stiffness of substrates through transmission of cell traction force (CTF) and respond through their cell morphology [9,10], cell migration [11,12] and stem cell fate [13–15]. Therefore, the transmission of CTF is a critical process to control cell-material interactions. An in-depth comprehension of CTF transmission from cells to biomaterials may help to guide the design of biomaterials capable of promoting tissue repair and regeneration.

Protein adsorption onto the surface of biomaterials generally occurs before cell adhesion [16,17]. As such, the adsorbed protein layer acts as a bridge between cells and materials. Materials regulate cell adhesion through regulating the status of adsorbed proteins [18,19] in a mode of bottom-up cell-material interactions. Once cells become adhered, the generated CTF will be sequentially transmitted to the protein layer [20] and then to the materials in a top-down mode, by which cells perceive the properties of both the protein layer and materials. The transition of CTF to the protein layer may partially unfold [20,21], reorganize [22,23], or even desorb [23–25] proteins such as fibronectin from the underlying substrates. These divergent protein phenomena by CTF are generally explained by the different cell-surface interactions resulting from protein compositions or substrate properties [26–28]. However, Altankov et al. [29] and Salmeron-Sanchez et al. [30] have noticed that the interaction strength between protein and substrate

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may influence the reorganization of adsorbed protein as well. Our recent work found that the adsorbed fibronectin (FN) molecules on $-NH_2$ are reorganized into fibres orienting along F-actins, yet those on $-OH$ surfaces are partially desorbed; further studies verified that the higher adsorption force of FN on $-NH_2$ than on $-OH$ was the main contributor [23]. These results provide an important hint that the adsorption force of a protein on substrates (F_{ad}) should be a critical mediator to the transmission of CTF.

Herein, we hypothesize that, F_{ad} could control the transmission of CTF from cells to protein layers and materials and further regulate stem cell fate. To test this hypothesis, mesenchymal stem cells derived from rat bone marrow (rMSCs) were cultured on stiff or soft poly(dimethyl siloxane) (PDMS) substrates of different F_{ad}. The effect of F_{ad} on the transmission of CTF to adsorbed fibronectin was visualized by immunostaining. In view of the fact that successful transmission of CTF to substrates may deform soft substrates [7], the effect of F_{ad} on the transmission of CTF to substrates were visualized by observing the deformation of soft PDMS. Finally, the perception and responses of rMSCs to the mechanical properties of substrates were evaluated by detecting their osteogenic and adipogenic differentiation on stiff and soft PDMS substrates of different F_{ad}, since the lineage specification of stem cells is known to be regulated by the mechanical properties of materials, such as stiffness [13,14,31]. The knowledge of how F_{ad} impacts cell-material interactions may provide biomaterial design with a diverse toolbox to control stem cell fate.

2. Materials and methods

2.1. Characterization of stiff and soft PDMS substrates with various F_{ad}

First, stiff and soft PDMS substrates were prepared by mixing the base and cross-linker of the PDMS kit (Sylgard 184, Dow Corning) in a ratio of 10:1 and 100:1, respectively. The base and cross-linker were mixed thoroughly, degassed and spin-coated (Spining Coater KW-4A, Chemat Technology, China) at 2000 rpm for 2 min onto glass slides. Then, the substrates were cured at 70 °C for 7 days. The stiff PDMS was labelled asPDMS-10 and the soft one as PDMS-100.

To fabricate PDMS with different F_{ad}, PDMS substrates with enriched surface $-OH$ and $-NH_2$ groups were constructed, since we have verified that the F_{ad} of FN on $-NH_2$ surfaces is much higher than on $-OH$ surfaces [23]. Before the construction of OH-enriched PDMS, intact PDMS substrates were first immersed in pentane for 24 h and acetone for 24 h to extract those uncured oligomers [32], which were then dried at 70 °C for 24 h. The obtained substrates were subjected to oxygen plasma for 25 s (Femto, Diener electronic, Germany), producing OH-enriched PDMS substrates. The selection of 25 s was to avoid significantly altering the topography and stiffness of PDMS induced by intensive oxygen plasma [33]. To get NH_2-enriched substrates, OH-enriched PDMS substrates were immersed in 10% solution of (3-aminopropyl) triethoxysilane (Sigma-Aldrich, USA) for 2 h [34]. The OH-enriched PDMS from PDMS-10 and PDMS-100 were labelled OH-10 and OH-100, respectively, and the corresponding NH_2-enriched PDMS substrates were labelled as NH_2-10 and NH_2-100.

To simulate substrates with extremely high F_{ad}, we further constructed PDMS with covalently bound FN molecules. To this aim, NH_2-10 and NH_2-100 substrates were dipped into 0.1 g/mL glutaric anhydride (GA, Sigma-Aldrich, USA) solution for 16 h, in EDC/NHS for 30 min, and finally in 20 μg/mL FN solution for 3 h at room temperature, producing FN-bound PDMS.

The surface chemistries of various PDMS substrates were characterized by utilizing a XSAM 800 photoelectron spectroscope (Kratos, UK) with an Al Kα X-ray source ($h\nu = 1486.6$ eV) under ultrahigh vacuum conditions (2×10^{-7} Pa). The binding energy scale was set with C–C/C–H bonds at 284.8 eV. The data analysis was carried out with a commercial software package (XPS PEAK, Version 4.1).

The static water contact angles were measured by the sessile drop method with a Model 200 video-based optical system (Future Scientific Ltd. Co., Taiwan) at room temperature and ambient humidity.

The Young's modulus and stress relaxation of PDMS were measured by compression tests of PDMS cylinders (15 mm in diameter, 2 mm thick). Stiff PDMS substrates were compress using a 4 mm diameter spherical tip and soft substrates using a 15 mm flat punch. All PDMS were equilibrated in PBS buffer prior to compression. The PDMS cylinders were compressed to 15% strain with a rate of 1 mm/min and the stress-strain curves were recorded. Young's modulus were calculated by analysing the obtained curves. Subsequently, the strain was held constant, while the stress was recorded as a function of time.

2.2. Adsorption of FN on various PDMS

Adsorption of FN was performed by immersing OH-10, OH-100, NH_2-10 and NH_2-100 substrates into 20 μg/mL FN solution for 2 h at 37 °C. The amount of adsorbed FN was quantified by using a microBCA Protein Assay Kit (Thermo Scientific, USA). The conformation of FN adsorbed on PDMS, represented by the cell binding domains in FN, was determined by enzyme-linked immunosorbent assay (ELISA) with HFN7.1 monoclonal antibody (Developmental Studies Hybridoma Bank, Iowa City, IA). The conformation of adsorbed FN on various substrates was also characterized by Atomic Force Microscopy (AFM, Icon Dimension with ScanAsyst, Bruke, USA) in a peak force tapping mode at room temperature. The images of the adsorbed FN were recorded with a ScanAsyst Air tip (Bruke, USA).

2.3. FN adsorption force on various PDMS

F_{ad} values on various substrates were detected by using a sphere/plane adhesion model. The experimental setup has been described previously by our work [23]. Briefly, human plasma FN (R&D Systems, USA) was covalently bound to NH_2-functionalized microspheres (diameter 18 μm, Aladdin, China) by using sulfo-succinimidyl 4-[*p*-maleimidophenyl]butyrate (Sulfo-SMPB) (Pierce, Thermo Scientific, USA) as a coupling agent (Fig. 2A, Fig. S2). The density and thickness of conjugated FN on microspheres were quantified by using a microBCA Protein Assay Kit (Thermo Scientific, USA) and AFM (Icon Dimension with ScanAsyst, Bruke, USA). The density was 3.81 ± 1.04 μg/cm^2, and the thickness was 2.67 ± 0.35 nm.

Subsequently, a PDMS substrate was mounted into a parallel plate flow chamber (PPFC), serving as the bottom of PPFC. The bubbles in PPFC were removed by filling the PPFC with PBS, and then FN-conjugated microspheres were slowly injected into the PPFC by a syringe. After 37 °C incubation for 2 h, the flow shear stress in the PPFC was increased step by step. At the end of each step, the microspheres remaining on the substrates were photographed by a phase contrast optical microscopy and counted by using the previous method [23]. The percentage of the remaining microspheres on the substrate was plotted as a function of the shear stress, within which the introduced shear stress capable of removing 50% of the initially adhered microspheres was denoted by $\tau_{50\%}$ as a critical shear stress. F_{ad} was calculated from $\tau_{50\%}$ according to the sphere/plane odel analysis reported previously [35,36].