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Effects of surface molecular chirality on adhesion and differentiation of stem cells

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ABSTRACT

Chirality is one of the most fascinating and ubiquitous cues in nature, especially in life. The effects of chiral surfaces on stem cells have, however, not yet been revealed. Herein we examined the molecular chirality effect on stem cell behaviors. Self assembly monolayers of L- or D-cysteine (Cys) were formed on a glass surface coated with gold. Mesenchymal stem cells (MSCs) derived from bone marrow of rats exhibited more adhering preference and thus less cell spreading on the L surface than on the D one at the confluent condition. More protein adsorption was observed on the L surface after immersed in cell culture medium with fetal bovine serum. After osteogenic and adipogenic co-induction at the confluent condition, a larger proportion of cells became osteoblasts on the D surface, while the adipogenic fraction on the L surface was found to be higher than on the D surface. In order to interpret how this chirality effect worked, we fabricated Cys microislands of two sizes on the non-fouling poly(ethylene glycol) hydrogel to pre-define the spreading areas of single cells. Then the differentiation extents did not exhibit a significant difference between L and D surfaces under a given area of microislands, yet very significant differences of osteogenesis and adipogenesis were found between different areas. So, the molecular chirality influenced stem cells, probably via favored adsorption of natural proteins on the L surface, which led to more cell adhesion; and the larger cell spreading area with higher cell tension in turn favored osteogenesis rather than adipogenesis. As a result, this study reveals the molecular chirality on material surfaces as an indirect regulator of stem cells.

1. Introduction

Cell-material interactions play a vital role in biomaterials, regenerative medicine, and other related fields [1–7]. While various pertinent cues have been investigated extensively [8–13], molecular chirality has not yet been paid much attention although stereospecificity is extremely important in Biology. For instance, life on earth always chooses the L-amino acids as the building blocks of proteins, rather than the D ones. Chirality sensation at interfaces in a living system is intrinsically related to sophisticated biophysical and biochemical processes. As the recognition of cells at chiral interfaces is concerned, Yavin and Yavin [14] first observed embryo cerebral cells of rats on surfaces coated by poly-L-lysine or poly-D-lysine, and found better cell adhesion on the L surface in 1974. Hanein and co-workers even observed a biased adhesion of kidney epithelial cells of *Xenopus laevis* on enantiomorphous crystal surfaces in 1994 [15]. After 10 min of incubation on the { 011 } faces of (*R*, *R*) and (*S*, *S*) crystals, the (*R*, *R*) surface was densely covered by cells, whereas only a few cells were observed on the (*S*, *S*) surface [15]. This result disclosed an interesting phenomenon that cells could sense the subtle difference of the enantiomorphous surfaces. About a dozen years later, Sun et al. [16] reported that the human neutrophil cells were more likely to adhere on the L-NIBC (N-isobutyryl cysteine) surface than on the D-NIBC surface. This adhesion bias on a stereospecific interface was enhanced by the increase of chiral centers on the interfaces [17–19]. El-Gindi et al. [20] even used L and D-penicillamines to enantiomerically functionalize zeolite crystals and found selective adhesion of endothelial and C-6-glioma cells on the D-penicillamine modified surfaces.

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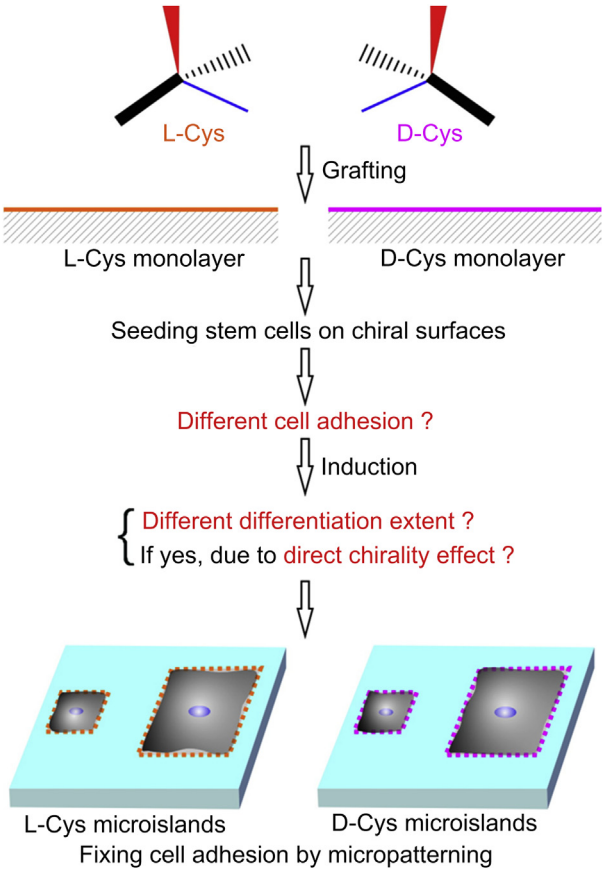

Fig. 1. Schematic presentation of the idea to explore the effects of chiral surfaces on the adhesion and differentiation of stem cells.

While it has been known that adhesion of some non-stem cells are affected by the surface chirality, the behaviors of stem cells on any chiral surface have not yet been reported. With the development of cell biology, biomaterials and regenerative medicine, stem cells have been recognized as a key cell type due to their ability of differentiation besides proliferation etc. [21–24]. The present paper is aimed to investigate the effects of the molecular chirality on stem cells. Both cell adhesion and differentiation will be examined.

We selected L- and D-Cys (cysteine) as a pair of chiral molecules. Their thiol groups were employed to form a self assembly monolayer (SAM) on a gold surface via the S–Au bond. Mesenchymal stem cells (MSCs) derived from bone marrow of rats were seeded onto the L or D monolayers. Besides checking the prior adhesion of MSCs on these chiral surfaces, we are more curious about whether or not the stem cell differentiation could be regulated by this chirality, as schematically presented in Fig. 1. If MSCs exhibit different behaviors of both adhesion and differentiation, we will further try to decouple the spreading area effect and the possible direct chirality-inducing effect on cell differentiation. So, we will fabricate micropatterns with the microislands of L- or D-Cys SAMs on an appropriate non-fouling background and localize single MSCs on those adherent microislands persistently. Stem cells will hence be examined in a highly controlled way.

2. Materials and methods

2.1. Preparation of L- and D-Cys SAMs

Clean cover glass (24 × 24 mm) was sputtered by gold on a SBC-12 sputter coater (10 mA for 1.5 min; KYKY, Technology Co., Ltd. China). The treated glass was ultrasonicated in absolute ethanol for 10 min, washed with deionized water (Milli-Q), and dried with nitrogen gas. The substrate was then immersed in 20 mM solutions of L-Cys (BioUltra) or D-Cys (Aldrich) in Milli-Q water, and kept at 50 °C overnight. The gold surfaces with SAMs of Cys molecules were rinsed with Milli-Q water (10 min × 3) to remove physically adsorbed Cys, and dried with nitrogen gas.

2.2. Contact angle measurements

In order to characterize the surface hydrophilicity before and after surface modification, static water contact angles on the untreated gold surface, L- and D-Cys modified surfaces were measured using the sessile drop method on a JC2000A optical contact angle meter (Powereach, China). For each group, $n = 6$.

2.3. X-ray photoelectron spectroscopy (XPS) characterization

XPS experiments were carried out in a RBD upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg K_α radiation (hν 1253.6 eV). The substrates with SAMs of L-Cys or D-Cys were pressed to a self-supported disk (10 × 10 mm) and mounted on a sample holder, then transferred into the analyzer chamber. The narrow spectra of Au and S with a high resolution were recorded to evaluate the L- and D-Cys grafting efficiency by using RBD 147 interface (RBD Enterprises, USA). Binding energies were calibrated by the containment carbon (284.6 eV with respect to C1s). Before and after immersing the substrates in cell culture medium containing 10 wt% fetal bovine serum (FBS) for 30 min, the narrow spectra of the N element were recorded to evaluate the relative amount of proteins adsorbed on the surfaces.

2.4. Preparation of L- and D-Cys microislands on poly(ethylene glycol) (PEG) hydrogels

Square microislands of gold with areas of 900 and 3600 μm^2 on glass were prepared using a traditional photolithography plus a lift-off technique. The gold microarray was then transferred from glass to the non-fouling PEG hydrogel, following our previous protocol [25,26]. In brief, the glass with a Au microarray was immersed in the ethanol solution of 1 mM linker, N, N′-bis(acryloyl) cystamine (Sigma) for 1 h, and then rinsed by ethanol 15 min × 3 times. The remaining linkers were bound to gold through the Au–S bond. Then, macromonomer poly(ethylene glycol) diacrylate (PEGDA, MW 700, Sigma) was used to prepare PEG hydrogels. Prior to polymerization, the photo-initiator 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (D2959, Sigma) was mixed with the macromonomers. The mixture was illuminated under UV exposure for 60 min, forming a PEG hydrogel. After peeling off the hydrogel, we eventually obtained the micropatterned surfaces with Au microislands on the non-fouling PEG background. The area of adhesive microislands was determined by the mask used at the stage of photolithography. To modify the gold microislands with L- or D-Cys, the gold micropatterns on PEG hydrogels were firstly washed with ethanol and deionized water (Milli-Q) successively, then immersed in 20 mM L-Cys or D-Cys aqueous solution, and kept at 50 °C overnight. Finally, the gold microislands chemisorbed with Cys were rinsed with Milli-Q water (10 min × 3 times) to remove physically adsorbed Cys.

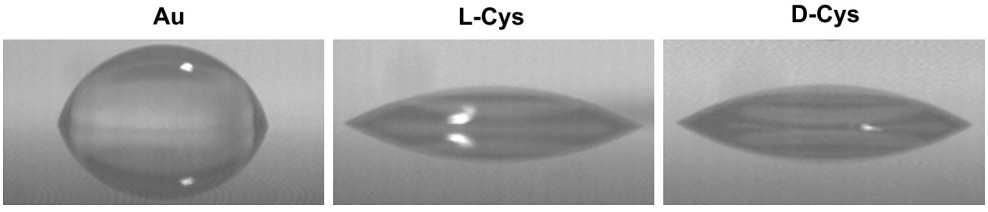

Fig. 2. Photographs of water droplets on surfaces in measurements of static contact angles before and after L and D-Cys grafting onto the gold surface.