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Stereospecific Interaction between Immune Cells and Chiral Surfaces

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The interaction between cells and extracellular matrix (ECM) substrate is an important factor governing the biocompatibility of materials and their successful applications as artificial implants and medical devices in the human body.[1] It is not only regulated by the ECM proteins[2] but also, more importantly, greatly influenced by the chemical and physical properties of the materials, such as the surface chemical compositions, wettability,[3] topographic structures,[4] etc. One of life's most distinctive biochemical signatures is its high selectivity for chiral molecular species,[5] such as helical DNAs, L-amino acids, etc., which play crucial roles in maintaining normal functions for living cells or organisms. As a result, many biological events are greatly influenced by the chirality of molecules. For example, enantiomers of the same compound may have very different tastes or smells and would exhibit different effects as drugs or plant growth regulators.[6] It points out the need to consider chiral interactions between cells and substrates. Such studies may not only help to understand the origin of the high stereoselectivity in life systems but also give interesting insight for the research of biomedical materials.

N-Isobutyryl-L(D)-cysteine (NIBC)[7] is an important biomolecule that has been extensively used in the chromatographic separation of chiral amino acids and is one of the few chiral mercaptanes commercially available with high purity. In order to address the above issue, gold sputtered surfaces modified with NIBC enantiomers were used as the model system, and their interactions with two important immune cells—macrophages and neutrophils—were investigated. Both cells exhibit distinct differences in the adhesion and activation behaviors on the different enantiomers' modified surfaces. This stereospecific result indicates a distinct biocompatible difference for the D and L surfaces, which points out a new direction to design the next generation of high-performance biomaterials.

Self-assembly[8] monolayers or multilayers have been widely applied in surface chemical modification and a wide variety of applications, including biosensing, molecular electronic devices, catalysis, etc., which have also been used as a model of ECM[9] in biochemical research. As shown in Figure 1, NIBC has the −SH group that can bond chemically with gold, which makes the cycteine and its derivatives able to form well-organized self-assembly layers on its surface.[10] Because the same treatment procedure was applied, it can be reasonably deduced that the two enatiomers' modified surfaces have the same chemical and physical properties, except for the mutually mirror-image arrangements of the NIBC molecules.

Macrophages are important immune cells in living bodies and perform various functions, such as migration, phagocytosis, secretion, antigen presentation, and survival through precisely modulated adhesion.[11,12] Their adhesion behavior was initially investigated on the NIBC enantiomers' modified smooth silicon substrates. Figure

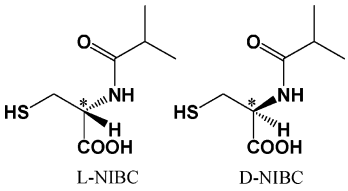

Figure 1. Molecular structures for NIBC enantiomers (∗= chiral center).

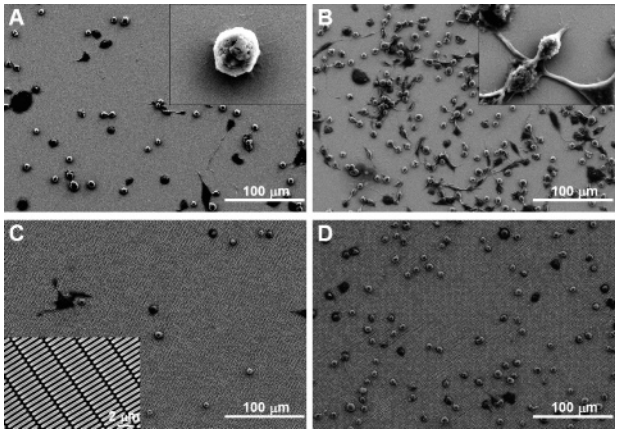

Figure 2. Macrophage adhesion on NIBC enantiomer-modified surfaces. (A and C) D-NIBC-modified surfaces; (B and D) L-NIBC-modified surfaces; (A and B) smooth substrates; (C and D) nanostructured substrates, the surface of which is composed of well-aligned nanoribbons with 360 nm widths, 3.6 μm length, and 720 nm height.

2A and B shows the typical scanning electron microscopic (SEM) images for the surfaces after 24 h of cell incubation. It can be noticed that the macrophages exhibit much different behaviors on the D (Figure 2A) and L surfaces (Figure 2B). First, the quantity of the adhered macrophages on the D surface [$(5.1 \pm 2.9) \times 10^4$ cm^{-2}] is much less (T test, $p < 0.01$) than that on the L surface [$(1.5 \pm 0.6) \times 10^5$ cm^{-2}], and second, the cell shapes on the two surfaces are different—about 47% macrophages remain separate with approximately round morphology (inset of Figure 2A) on the D surface, while this value decreases to only about 26% on the L surface, and most cells have extruded the pseudopods or gathered together to show a highly spreading fashion (inset of Figure 2B). Interestingly, the control experiment on the bare gold sputtered silicon substrate gives an intermediate result for both the adhesion quantity [$(1.3 \pm 0.3) \times 10^5$ cm^{-2}] and the cell shape. These results show that the cell behaviors are greatly influenced by the surface modification, and the D-NIBC modification can decrease the macrophage adhesion on the ECM substrate, while the L-NIBC has the opposite effect.

Since the surface micro- and nanostructures are also important factors influencing the cell/substrate interaction,[4] in order to confirm the above phenomenon, we conducted the same experiments on a

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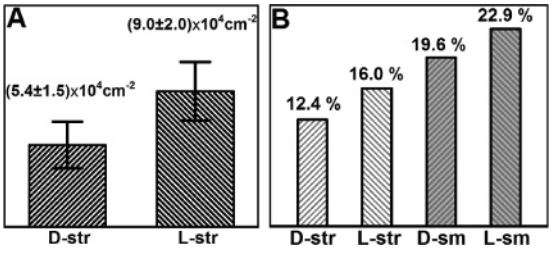

Figure 3. Neutrophil adhesion and activation on the enantiomeric surfaces. (A) Quantities of the adhered neutrophils on material surfaces by SEM observation. (B) Percentages of the activated neutrophils by immunofluorescence experiments and flow cytometer analysis, in which CD11b and CD62L antibodies were used as markers and the $CD11b^+/CD62L^+$ events show the activation of neutrophils. D-sm, L-sm: smooth silicon substrates with D- and L-NIBC modifications; D-str, L-str: nanostructured PDMS substrates with D- and L-NIBC modifications.

prestructured poly(dimethyl siloxane) (PDMS) substrate with a well-aligned nanoribbon pattern (inset of Figure 2C). Figure 2C and D shows the macrophage adhesion result on these substrates with gold sputtering and the D- and L-NIBCs' modification, respectively. Similar to the results of our previous work,[4a] where largely suppressed platelet adhesion and activation on the aligned carbon nanotube substrates were observed, macrophages also exhibit much less adhesion on the nanostructured substrates for both the chiral surfaces, and most of the adhered macrophages maintain a roughly round shape. Comparison of Figure 2C and D reveals a phenomenon of stereospecific cell adhesion that is the same as that of the smooth substrates, and the quantities of the adhered macrophages on D and L surfaces are $(1.3 \pm 0.7) \times 10^4$ and $(5.8 \pm 3.1) \times 10^4$ cm^{-2} (T test, $p < 0.01$), respectively. These results indicate that surface chirality has great influence on the cell/substrate interaction, although the other chemical and physical properties of the surface remain the same.

In order to examine whether the stereospecific effect is a unique property of cell type, we also checked the cell/substrate interaction using another important immune cell—neutrophil. The cells from a healthy volunteer were seeded on the enantiomeric surfaces, and their adhesion and activation behaviors were investigated after about 0.5 h of incubation. Figure 3A shows a comparison between the numbers of the adhered neutrophils on the nanostructured chiral surfaces (for the smooth substrates, almost no neutrophil adhesion can be observed on both the D and L surfaces; it may be caused by the special adhesive mechanism of the neutrophils, which makes them unable to grasp the surface when it is too smooth for the cells). On the D surface, the quantity of the adhered neutrophils is about $(5.4 \pm 2.0) \times 10^4$ cm^{-2}, which is less than 2/3 (T test, $p < 0.01$) of that on the L surface [$(9.0 \pm 2.0) \times 10^4$ cm^{-2}]. On the other hand, the immunofluorescence experiments and flow cytometer analysis (Figure 3B) show an activation level to the neutrophils ($CD11b^+/CD62L^+$ events) for the D surfaces that is distinctly lower than that on the L surfaces [19.6 and 22.9% (X^2 test $p < 0.005$) for the smooth substrates, 12.4 and 16.0% (X^2 test $p < 0.005$) for the nanostructured substrates], which is well consistent with the adhesion result.

In a previous work,[13] Adaddi et al. also reported the differential behaviors of epithelial cells on the enantiomorphous calcium tartrate crystal surfaces. It indicates that the stereospecific interaction between the cells and the ECM substrate is not a unique but a common effect that is applicable to different cell types and materials. The less adhesion and activation of macrophages and neutrophils on the D enantiomer modified surfaces than on the corresponding L surfaces shows that the D surfaces may help to decrease the immunological reaction or rejection by living bodies, and thus they are more biocompatible. It is thus considered that the design of surface chirality may bring a novel direction for the biomaterial design, which is well complementary to the existing strategies to improve the biocompatibility.

The recognition, adhesion, and activation of cells on artificial substrates are complex processes that need the cooperation of various extracellular and intracellular proteins, which includes both a complex intracellular signaling cascade and a physical or chemical interaction of extracellular components with cell surface proteins and an interaction of closely related cell surface antigens.[14] Because of the highly chiral selectivity of the natural amino acids that constitute the proteins, as well as other important biomolecules, including DNA, RNA, etc., it could be reasonably speculated that the surface chirality may be recognized by the cells through special chemical interaction[5,15] between the chiral moieties of the surface and the cells, which may release different signals to the cells and result in different cell/substrate interaction.

In conclusion, the surface chirality has great influence on the cell/substrate interactions. In-depth investigations of this effect may not only bring interesting insight to the design of novel high-performance biocompatible materials but also help to better understand the high selectivity of chirality in biosystems. This effect may also bring novel application insights for the NIBC and other chiral biomolecules.

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Supporting Information Available: Experimental details and flow cytometer analysis results for neutrophils. This material is available free of charge via the Internet at http://pubs.acs.org.

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