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Micro-textured stainless steel material towards enhancement for adhesion of red blood cell

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Abstract

In this research, the stainless steel sheet (EN-1.4301) is selected to investigate the adhesive interaction of the RBC to the textured and non-textured surfaces. The sheet sample is equally divided into two sides, one is textured with pillar patterns and the other side remains non-textured as conventionally finished surface (smooth surface). The pillar patterns were fabricated by laser surface texturing (LST) technology with a picosecond laser. Atomic force microscopic (AFM) single-cell indentation tests are performed on the RBC to the counter-surfaces. Based on the experimental results, Force-Piezo Displacement curves under all three press distances (30, 50, 80%) of the RBC thickness, show strong adhesion signals of the RBC to pillar textured stainless steel surface. Compared to non-textured stainless steel surface, such adhesive behavior is not observed. From the perspective of contact mechanics, these phenomena can be explained based on the change of bending energy, interaction energy and surface contact area of cell membrane due to the micro-deformations among the contact regions of the RBC and pillar patterns. These findings expand our understanding of RBC adhesive behavior (possibly to all cell types) to the micro-textured counter-surface.

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1 Introduction

Stainless steel is a common metallic material which often implanted in human body as biomaterial. Compared to other materials like ceramics and polymers, stainless steel obtains superior tensile strength, fracture toughness and fatigue behavior, also, relatively better corrosion and wear resistance compared to materials like ceramics and polymers (Muley et al. 2016). However, the successful implantation of stainless steel biomaterials unconditionally encounters interaction with cell, e.g., red blood cell (RBC). According to the literature, RBC is one important cell with a major function in transport of oxygen and regulation of blood flow (Bain 2017). Thus, it is crucial to investigate the adhesive interaction between the stainless steel and red blood cell which is the most important cell in human body.

The interaction between RBC and stainless steel is a complex micro-mechanics initiates with attachment on the counter-body and RBC's shape deformed from biconcave disk to flat during the process. From the perspective of contact mechanics, such interaction is exhibited through the formation of adhesion (Ivanova et al. 2017). The structure and material properties of RBC have been investigated with a great amount of studies. The mechanical properties, e.g., elasticity, membrane tension, cell

shape, etc. have been investigated with different techniques. The most popular ones are: optical tweezers, magnetic beads, micropipette aspiration and atomic force microscopy (Li et al. 2014; Butt et al. 2005; Ishihara et al. 2017; Hogan et al. 2015). Among these techniques, modern atomic force microscopy (AFM) has the advantages of high spatial resolution and force sensitivity, therefore, it is the most suitable method to study the micro- and nano-scaled structures including living cells like RBC. Optical tweezer, magnetic beads and micropipette have limitations in weak applied force (< 150 pN) and short pulling range.

In this research, the adhesive interaction between cell and stainless steel is investigated. In addition, the surface topography of the stainless steel sheet is modified by laser surface texturing technology (LST). A deterministic texture (pillar structure) is designed and fabricated on the half side of stainless steel sheet, together with stochastic (non-textured) surface as reference on the other half side, both sides are conducted in the atomic force microscopy-based single-cell indentation tests.

2 Materials and methods

2.1 RBCs preparation and stainless steel samples fabrication

RBCs were taken from healthy adults on the day of experiment by pricking fingertip. In addition, cell samples were centrifuged (Eppendorf AG) three times in phosphate-buffered saline buffer (PBS, GE life sciences) at 3000 r/min for 75 s each time to remove the leukocytes, plasma and platelets.

The stainless steel sheet material (EN-1.4301) is selected as the testing sample with dimension of 10 mm $\times$ 4 mm. The sample is divided into two sides, one is textured with pillar patterns and the other half side remains non-textured as conventionally finished surface (smooth surface with surface roughness, R_a , of 0.13 μm). The pillar patterns were fabricated by laser surface texturing (LST) technology with a picosecond laser. The diameter of each pillar is 2 μm and depth of 5 μm .

2.2 SEM sample preparation

Cantilever with attached single RBC was treated by 2.5% glutaraldehyde in PBS for 10 min. Then, the RBC was gradually dehydrated by 70–100% ethanol in MilliQ water for 10 min each time. The treated RBC on probe was kept in shape when it was moved to the chamber of SEM.

2.3 Experimental system design

The experimental system is a scanning probe and inverted hybrid microscope, including an atomic force microscope (CSPM4000, Being Nano-instruments, Ltd.), an inverted optical microscope (GX41, Olympus) and ultra-high precision positioner in 3 directions (KXAV1010, Zolix). The detector with probe of AFM is positioned above the lens of inverted microscope and the sample is placed on the piezo which could move with the movement of ultra-high precision motorized positioner (refer to Fig. 1). This experimental system makes it possible to visually adjust relative horizontal position between probe and sample or cells.

2.4 Cell probe preparation

With the assistance of the scanning probe and inverted hybrid microscope, the AFM probe (HQ:CSC38/Tipless with the spring constant on the order of 0.09 N/m, MikroMasch) was immersed into a drop of Corning Cell-Tak (Corning Co.) in 5% acetic acid. Cell-Tak is a strong tissue adhesive consisting of marine mussel proteins. The binding is of nonspecific nature and is assumed not to activate RBCs. After 20 min incubation in room temperature, the acetic acid evaporated and a layer of Cell-Tak left behind on the cantilever. After washing with ethanol and PBS, the cantilever was ready to attach RBC.

To convert the units of measurement volts to meaningful result in the units of newton. The system sensitivity and specific spring constant of cantilever have to be determined. The system sensitivity is determined from a force–displacement curve recorded by pressing the cantilever without cell on a hard surface (i.e. glass). The specific spring constant of the cantilever without cell is determined by thermal tune method.

The attachment procedure took place in a PBS solution. To attach a RBC to the cantilever, the latter was lowered manually by an ultra-high precision motorized positioner until a preset cantilever deflection was reached (i.e., the preset force point $F_{\text{set}} = 0.001$ μN), indicating the contact between the cantilever and the cell. Afterwards, the cantilever was withdrawn continuously at low speed until the cell was no longer in contact with the bottom surface of liquid cell. At this point, it is essential that the adhesion of the RBC to the Cell-Tak needs to be greater than the adhesion of the cell to the bottom surface of liquid cell. After the cell was attached to the cantilever, the cell was placed above stainless steel sample that laid on the bottom of liquid cell.

To avoid any further sources of measured interactions (e.g., interactions of stainless steel sample to the Cell-Tak coated surface of cantilever), 0.1 g/dl bovine serum

Fig. 1 Schematic figure of AFM single-cell indentation tests

albumin (BSA, Sigma-Aldrich) was added to the solution after attaching the RBC to the cantilever by pipetting several drops of BSA to the solution. BSA can passivate the surfaces of Cell-Tak coated cantilever and make investigated interactions to be purely physical (i.e., we assume there are no adhesion proteins are involved into the measured interactions that could possibly be blocked by the incorporation of BSA into the RBC membrane) (Steffen et al. 2013).

3 Results and discussion

Based on the experimental results, the adhesive interaction between RBC and the textured surface is clearly observed. According to the Force-Piezo Displacement curve shown in Fig. 2, under the press distance of 30% of the RBC thickness (approximately 0.6 μm) with the time interval of 1.3 s, the retract line (indicated as blue line) of the RBC to textured surface have noticeable fluctuations compared to the approach line (indicated as red line). The same result also occurs when the press distance increased to 50% of the RBC thickness (approximately 1 μm) with the time interval of 2.2 s. Moreover, when the press distance reached 80% of RBC thickness (approximately 1.7 μm) with the time intervals of 3.4 s, right before the RBC dispatch from the textured surface, a more intense zigzag signal is shown

in the Force-Piezo Displacement curve. In other words, these fluctuations of retract lines illustrate the rebound delay during the lifting process of cantilever, indicating that the adhesion exists between the RBC and textured surface. Furthermore, the deeper press distance of the RBC, more intense rebound delay is observed. Such delay is caused by the adhesive interaction between the cell membrane and the surface structure of counter-body. On the contrary, the reference side (non-textured smooth surface) shows no signs of rebounding. After the completion of adhesion tests, the RBC is examined along with the AFM cantilever by SEM. According to the Fig. 3, the RBC is intact with no signs of damage.

To better understand the cause of this phenomenon, a schematic diagram is illustrated in Fig. 4. Cell adhesion heavily depends on the mechanical property of RBC's membrane, and numerical studies show favorable prediction of the RBC with nonlinear elastic behaviour (Chee et al. 2008; Xu et al. 2013). The Hertz model is widely used for an elastic material in contact with a spherical shaped counter-body, in this case, assuming a RBC is a homogeneous and isotropic material and the contact mechanics of RBC is described as (Guz et al. 2014):

$$F(i_c) = \frac{16}{9} E^* \sqrt{\frac{R_{\text{pillar}} + R_{\text{RBC}}}{R_{\text{pillar}} + R_{\text{RBC}}}} i_c^{3/2} \quad (1)$$

Textured Sample

Non-textured Sample

(a)

(b)

(c)

(d)

Fig. 2 **a** The SEM images of textured and non-textured surfaces; **b** Force-Piezo Displacement curve of RBC and samples of **b** press distance = $0.6 \mu\text{m} \approx 30\%$ RBC thickness, **c** press distance = $1 \mu\text{m} \approx 50\%$ RBC thickness, and **d** press distance = $1.7 \mu\text{m} \approx 80\%$ RBC thickness

Fig. 3 SEM image of the RBC after AFM single-cell indentation tests

where F is the applied load; E^* is the composite Young's modulus ($\frac{1}{E^*} = \frac{1-\nu_1^2}{E_1} + \frac{1-\nu_2^2}{E_2}$; E_1 , E_2 and ν_1 , ν_2 are the Young's modulus and Poisson's ratios for stainless steel sheet and RBC respectively); i_c is the indentation displacement; r is the radius of RBC; and R_{pillar} is the radius of single pillar structure.

From the perspective of contact mechanics, assuming that no adhesion proteins are involved into the measured interactions (refer to Sect. 2.4), cell membrane is deformed in contact with the pillar patterns when the external force is applied on the RBC. This deformation is manifested as micro-indentation, and the region in contact is bent under the pressure. The bending energy is an important mechanical parameter to predict the buckling of a cell membrane, according to the neo-Hookean model, it can be defined as following (Tan et al. 2010):

$$k_b = \frac{1}{3} \mu_{neo-Hookean} h_0^2 \quad (2)$$

where k_b is the bending energy of RBC membrane; $\mu_{neo-Hookean}$ is the initial shear modulus of RBC membrane for neo-Hookean model; h_0 is the initial thickness of RBC membrane. The bending energy of RBC membrane is

Fig. 4 Schematic diagram of contact between cell membrane and **a** pillar textured surface, and **b** non-textured surface

investigated in other literature, and the known range is 1.7×10^{-19} to 7×10^{-19} (Sleep et al. 1999).

As the result, the elastic cell membrane fills the voids and pores of the textured counter-surface and firmly bound (refer to Fig. a). In this way, the surface contact area is increased due to the combination area of tips, sides and valleys of the pillar structures. With consideration of both the bending energy and the surface area of RBC membrane, the adhesion strength can be described as (Ziherl and Svetina 2007):

$$\zeta = \frac{\Gamma A_0}{8\pi k_b} \quad (3)$$

where A_0 is the surface area of RBC; Γ is the interaction energy; k_b is the bending energy of RBC.

However, when the RBC is in contact with smooth surface (non-textured stainless steel sample), the contact region of counter-surface and RBC membrane is relatively flat. Without sufficient deformation, the adhesive interaction between the RBC and non-textured stainless steel surface is limited. Overall, we can conclude that adhesive interaction of RBC to stainless steel material can be manipulated by modifying the surface texture, e.g., pillar structure.

4 Conclusion

In this research, the stainless steel material is selected to investigate the adhesive interaction of the RBC to pillar textured and non-textured surfaces. The Force-Piezo Displacement curve from AFM single-cell indentation tests show, under all three press distances (30, 50, 80%) of the RBC thickness, the RBC demonstrates strong delay signals to pillar textured stainless steel surface when the RBC attached cantilever is moving away from the counter-surface. Compared to non-textured stainless steel surface, such rebound delay is not observed, indicates no significant adhesive interaction is produced. From the perspective of contact mechanics, these phenomena can be explained based on the change of surface contact area of cell membrane due to the micro-deformations among the contact regions of the RBC and pillar patterns.

These findings expand our understanding of RBC adhesive behavior (possibly to all cell types) to the micro-textured counter-surface. In the future, other influential

factors, e.g., different geometric structure, parameters, contact angles, etc. of micro-texture counter-surface to cell adhesion can be further investigated.

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