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Shear assay measurements of cell adhesion on biomaterials surfaces

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ABSTRACT

The paper examines the adhesion of human osterosarcoma (HOS) cells to selected biomaterials surfaces that are relevant to implantable biomedical systems and bio-micro-electro-mechanical systems (BioMEMS). The four biomaterials that were explored include: silicon, silicon coated with a nanoscale layer of titanium, Ti–6Al–4V, and poly-di-methy-siloxane (PDMS). The interfacial strengths between the HOS cells and the biomaterials surfaces were determined using a shear assay technique. The adhesion forces were determined using a combination of confocal microscopy images of the three-dimensional cell structure, and computational fluid dynamics (CFD) simulations that coupled actual cell morphologies and non-Newtonian fluid properties in the computation of the adhesion forces. After cell detachment by the shear assay, immunofluorescence staining of the biomedical surfaces was used to reveal the proteins associated with cell detachment. These revealed that the nano-scale Ti coating increases the cell/surface adhesion strength. Silicon with Ti coating has the strongest adhesion strength, while the other surfaces had similar adhesion strength. The measured strengths are shown to be largely associated with the detachment of focal adhesion proteins from extra-cellular matrix (ECM) proteins.
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1. Introduction

The adhesion between biological cells and biomaterials is most important for the integration of biomedical implants, and wound repair around implanted biomedical system [1]. These include orthopaedic/dental implants and implantable Bio-Micro-Electro-Mechanical System (BioMEMS). In such systems, weak interfacial adhesion may give rise to micro-motion and long term degradation of the interfaces that are formed.

Due to the significance of the cell/surface adhesion, a number of researchers [2–10] have developed different techniques for the measurements of cell adhesion. Atomic force microscopy (AFM) has been used to measure the ligand-receptor bonds associated with cell/surface adhesion. By coating special proteins on AFM tip, adhesion forces with pico-Newton magnitudes have been measured for specific ligand-receptor interactions [11–13].

Zhang and Moy [10] have quantified the detachment force of streptavidin–biotin by the downward deflection of the cantilever. Li et al. [6] have also measured the dynamic response of $\alpha 5\beta 1$ integrin–fibronectin to a pulling force, and the single molecule rupture force was measured between live K562 cells and fibronectin. Although AFM methods provide molecular level measurements of adhesion, it is clear that the micro-scale dimensions of cells require measurements at the micro-scale.

Huang et al. [5] have used a glass beam (75 μm in diameter) as a cantilever to contact and detach single chondrocytes from glass slides. The detachment force after 6 h of cell culture was reported to be 388 ± 78 nN. Optical tweezers [7,9] and micropipette methods [2,4,8] have also been widely used to measure the adhesion strength. However, all of these methods result in "hard" contacts with cell surfaces. They may, therefore, induce additional biological responses from the cells.

Compared to the "hard" contact methods, hydrodynamic forces exerted by fluid flow can softly detach cells from biomedical surfaces. Thus, under fluid flow conditions, the applied hydrodynamic forces are less likely to induce additional biological responses that may compromise the measured strength data. Centrifugation [3,14] and rotating plates [15,16] have also been used widely to measure the adhesion strength. However, the *in-situ* observations of living cells are difficult during such measurements.

Hence, there has been increasing interest in the use of the shear assay method for the "soft" detachment of cells from biomedical substrates. This has been done largely using a parallel flow chamber [17–21] that permits the *in-situ* observation of cell deformation and detachment during (cell culture) fluid flow across cells in a microfluidic channel. In this way, the onset of cell detachment can be determined, which can be monitored with *in-situ* microscopy.

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In most cases, cell detachment stress during shear assay measurement has been measured using a simple boundary layer equation that only counts for the wall shear stress [17,21,22]. However, this does not adequately account for the cell shape. Hence, numerical simulations have been used by Goldstein and DiMilla [23] to simulate the effects of cell shape on the detachment of murine 3T3 fibroblasts from self-assembled monolayers. These simulations, in which the Navier Stokes equations have been solved numerically, have shown that cell shape should be considered in the modeling of fluid flow over cells.

Adhesion of circulating leukocytes [24–28] to the vascular endothelium is another typical cell/surface interaction within shear flow environment, where cell shape is important for adhesion. Circulating leukocytes bind to the wall by adhesion molecules, and show a characteristic 'stop-and-go' motion of rolling. More and more attentions have been put on the 'tear-drop' shape of adherent leukocytes under shear flow [29–32]. Compared to rigid ball shape, the 'tear-drop' shape may significantly [29,31] reduce the shear stress and the fluid drag acting on the leukocytes, and hence increases the lifetime of the selectin bonds. Therefore, cell shape plays a key part in accurately estimating the flow resistance and fluid drag, and then understanding the mechanics of cell adhesion in the microenvironment surrounding the cells.

Shear stress also causes deformation of cyto-skeleton filaments and stress concentration on focal adhesions [33–35]. The effects of shear stress were also carefully studied for the binding between integrin and extracellular matrix [36,37], as well as cell mechanics [38]. Those studies revealed that the global activation of shear-forces by fluid flow is transported to local discrete focal adhesion locations in cells. Thus, there is a need to explore cell/surface adhesion strength measured by shear flow, and the connection between the overall adhesion strength and the detailed cellular focal adhesion on substrates.

This paper presents the results of a combined experimental and computational study of the shear assay measurement of the interfacial strengths between human-osteosarcoma (HOS) cells and biomedical surfaces that are related to orthopedics/dentistry and BioMEMS. Following the introduction, the materials and experimental methods for the study of cell spreading and adhesion are presented in Section 2. This is followed by Section 3, in which the shear assay model is described. The results and discussion are then presented in Section 4 before summarizing the salient conclusion arising from the current work in Section 5.

2. Materials and experimental methods

2.1. Materials and substrate preparation

The materials that were studied in this work include: (100)-oriented single crystal silicon, titanium-coated silicon, Ti–6Al–4V, and poly-di-methyl-siloxane (PDMS). The silicon was chosen as a representative material that is used in BioMEMS and implantable microelectronics [39–41]. It was coated with a nanoscale thickness of titanium to explore possible effects of titanium, which is a cytoactive, biocompatible material that has been shown to elicit no cytotoxic reactions in the body. Similarly, the Ti–6Al–4V was chosen as a representative cytoactive bioactive material that is used in orthopedics and dentistry, while the PDMS was selected as the materials of choice for soft implantable BioMEMS and microelectronics.

The silicon that was used in these experiments was obtained from Silicon Valley Microelectronics in San Jose, CA. The silicon wafers were obtained in the form of n-type, 100 mm, 375 μm thick, single crystal Si (100) wafers. The wafers were cut into samples that were either 5 mm × 5 mm or 35 mm × 35 mm in area. These were used, respectively, for cell spreading and shear assay experiments. They were then cleaned by initial sonication in acetone for 30 min. This was followed by boiling in 1:3 H_2SO_4:H_2O_2 for 45 min. Following an extensive rinse in double distilled water (dd H_2O), the samples were boiled in a 1:1 HCl:H_2O_2 solution. They were then given a final rinse in dd H_2O and sterilized in ethanol.

Half of the silicon samples were coated with a 50 nm layer of titanium. This was deposited using a Denton DV-502A e-beam evaporator (Denton Vacuum, Moorestown, NJ).

The Ti–6Al–4V substrate was fabricated by forging at Wyman Gordon, Houston, TX. Samples surface was mechanically polished using colloidal silica suspension. A multi-step washing procedure was then used for the passivation. This was designed to remove oils, debris, and dirt and produce a consistent titanium oxide layer (typically 2–10 nm thick) on the surface. The washing procedure included sonication in distilled water/detergent for 20 min; rinsing 3 times in distilled water; sonication in acetone for 20 min; passivation in about 30% nitric acid for 15 min; rinsing in dd water and sterilization in 100% ethanol for 30 min, before drying with nitrogen gas.

The PDMS was made by mixing 10 parts of 184 silicone elastomer (Dow Corning, Germantown, WI) base with 1 part of curing agent. The mixture was then cured at 60 °C for 12 h. Regular dd water cleaning and ethanol sterilization were used before cell culture.

2.2. Surface characterization using atomic force microscopy

Since surface topography has been shown to have a strong influence on cell spreading and adhesion [42], a special effort was made to characterize the roughness of each substrate after surface preparation. This was done using a Digital Instruments Nanoscope III Atomic Force Microscope (Veeco Instruments, Woodbury, NY). The surface roughness was operated in the tapping mode. The scanning area was set as 10 μm × 10 μm, and at least five different locations were measured to obtain an accurate representation of the surface roughness of each substrate employed in this work.

2.3. Cell spreading

In order to study the initial spreading and attachment of HOS cells to the selected surfaces, 48 hour cell culture experiments were performed. The Human Osteosarcoma (HOS) cells were obtained from American Type Culture Collection in Manassas, VA. Prior to seeding, the HOS cells were maintained in 25 cm^2 cell culture flasks at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM) (Quality Biological, Gaithersburg, MD) containing 10% Fetal Bovine Serum (Quality Biological, Gaithersburg, MD), 5% Amphotericin and 5% Penicillin/Streptomycin (Quality Biological, Gaithersburg, MD).

A cell suspension was then prepared as follows. A flask of cells was rinsed using 2 mL of a 0.25% trypsin solution diluted in Dulbeccos Phosphate Buffered Saline without Ca^{++} or Mg^{++}. This solution was immediately aspirated from the flask to remove surface proteins. An additional 2 mL of trypsin was added and the flask was allowed to incubate for several minutes in order to release the bonds attaching the cells to the surface.

After incubation, the flask was agitated to free the cells. Subsequently, 5 mL of serum-free DMEM was added to the trypsin/cell solution and the entire mixture was removed to a 15 mL conical tube. The tube was then placed in a centrifuge for 3 min at 3500 rpm (RPM). The supernatant was aspirated out and an additional 5 mL of serum-free media was added to the tube. The cells were then re-suspended through gentle pipetting until the solution appeared uniform. After re-suspending the cells, the suspension was pipetted onto the samples, which were then incubated at 37 °C for their allotted periods of time.

2.4. Confocal microscopy

Confocal microscopy was used to obtain three-dimensional images of cell structure and shape. To facilitate imaging with a confocal microscopy, the cells were dyed using cell tracker (Invitrogen Corporation, CA). The molecular probe was first diluted into serum-free cell culture

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