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Measurement of the tensile strength of cell–biomaterial interface using the laser spallation technique

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Abstract

A previously developed laser spallation technique to determine the tensile strength of thin film interfaces was successfully adopted to determine the tensile strength of interfaces between three different live mammalian cells (osteoblast, chondrocyte and fibroblast) and polystyrene (untreated and fibronectin coated) and titanium surfaces. No noticeable differences in the interfacial tensile strength values were found across the three cell types on the same substrate although osteoblasts showed slightly lower adhesion strength when cultured on untreated polystyrene surfaces. Significant differences were, however, measured for cells treated on different surfaces. Use of fibronectin increased the interfacial tensile strength for all cell types, and cells bonded much better to titanium than to untreated polystyrene surfaces. Cell interfacial strength was higher when cultured with serum than in a serum-free environment. The results demonstrate the remarkable sensitivity of the laser spallation experiment in determining the effects of local interfacial microstructure and chemistry on cell adhesion.

Keywords: Cell adhesion; Laser spallation experiment; Tensile strength; Osteoblast adhesion; Fibroblasts

1. Introduction

It is well known that the differentiation, proliferation, migration and function of anchorage-dependent animal cells depend upon the chemical and physical properties of the substrate surface. Consequently, depending upon the final application, biomaterial surfaces can be engineered to invoke a desired cell response. For example, materials designed for heart valve and blood vessel applications require minimal cell adhesion, whereas other applications such as scaffolds designed for tissue engineering of bone require the cell adhesion to be maximum. One of the prerequisites for surface engineering is the availability of an adhesion metrology tool that not only provides a quantitative measure of true adhesion but is also sensitive to the microstructural details of the interfacial region that are controlled by the variables associated with a substrate's surface and cell structure. This paper introduces a novel cell adhesion metrology tool with such characteristics.

Several types of cell adhesion assays are currently used to provide a wide range of information about cell adhesion. Each technique is associated with unique strengths and drawbacks. Rinsing techniques have been used to evaluate cell affinity for a substrate by reporting the ratio of adherent cells remaining on a surface to total cells plated after a detachment force has been applied [1]. These methods provide a gross evaluation of cell affinity between surfaces of interest and are efficient for testing large numbers of cells on a variety of surfaces. However, data is largely qualitative as interfacial strengths can rarely be determined with this method. Other limitations of rinsing assays include

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limited reproducibility of forces applied to detach cells. Methods that quantitatively evaluate cell adhesion strength have been developed and fall broadly into the categories of hydrodynamic, centrifugation and micromanipulation techniques.

Hydrodynamic techniques designed to measure cell adhesion strength include the spinning disk technique [2,3], parallel plate apparatus [4,5], radial flow chambers [6,7] and jet impingement [8,9], among others. For each of the methods listed above, a large population of cells is simultaneously exposed to a range of shear stresses during a single experiment. This is desirable because large test populations reduce experimental variability and increase the statistical significance of the results. In most cases, hydrodynamic techniques measure only the shear force required for cell detachment. The drawbacks to some hydrodynamic techniques include possibility of cell rupture during detachment, resulting in a measurement that contains cohesive as well as adhesive components [9]. Considerable local deformation also occurs during detachment, which may result in an inaccurate calculation of adhesion strength.

Centrifugation assays use a centrifuge to apply a normal force to detach adherent cells [10]. Forces are reproducible, and large numbers of cells can be easily tested on a variety of surfaces. The limitations of centrifugation assays include relatively low detachment forces best suited for short-term culture conditions (<60 min) or weak cell–substrate interactions. Centrifugation assays measure only a single force per experiment, so multiple tests must be completed to determine the threshold adhesion strength.

Micromanipulation techniques include a variety of experimental assays including micropipette suction, atomic force microscopy (AFM) and microcantilever probing. Micropipette suction is used to gain quantitative information about cell attachment strength for individual cells, and therefore average adhesion strengths gathered using this technique represent a much smaller cell population. AFM assays are designed to study single molecular interactions. However, current concepts suggest that bond breakage may be governed by a series of transition states and no single force for bond breakage exists [5]. Additionally, AFM systems are designed to study monovalent interactions when in practice cell adhesion is mediated by multivalent interactions which can lead to receptor aggregation and trigger further response within the cell [11]. Micromanipulation assays may be slightly more versatile than flow techniques due to their ability to measure the normal or shear forces required to detach cells from a substrate depending on the experimental mode. Some complications with micromanipulation systems arise from the direct probe–cell contact which causes rapid deformation and material property changes throughout the duration of the experiment [12]. This directly affects cell adhesion.

In addition to above, in almost all of the above techniques, the cell interface is subjected to a multiaxial stress-state. No direct measurement of this stress-state at the cell detachment point is accomplished or even possible in these techniques. Instead, the local stress-state is computed using simple or sophisticated finite-element-based models that use experimentally measurable far-field flow parameters (in jet impingement and radial flow chamber-based techniques) or force/deflection characteristics (in microcantilever-based techniques) as an input. Since the material deformation in these experiments is complex, any reasonable model will involve largely unknown deformation properties of the intervening medium. Nevertheless these techniques provide a valuable measure of relative adhesion. Unfortunately, such an indirect measurement of adhesion disallows any connection of the far-field parameters to atomic-level cell adhesion processes, as needed for developing tailored biomaterial surfaces or accomplishing the goals set above.

In this study, a laser spallation technique that was previously modified [13] to measure the attachment strength of MC3T3-E1 preosteoblasts on various types of polystyrene surfaces was used to measure adhesion of additional cell types (osteoblast, chondrocyte and fibroblast) and substrates (untreated and fibronectin-coated polystyrene and titanium substrates). The effect of culturing the cells in serum and serum-free conditions on resulting cell adhesion strength was also investigated. The selected cell types are well characterized for in vitro orthopedic applications. Polystyrene is a material commonly used for cell culture and was chosen as a baseline material, while titanium is a biomaterial commonly used clinically for orthopedic and prosthedontic implants. Fibronectin, a widely studied adhesive protein, was chosen to study the sensitivity of the laser spallation technique to biologically relevant surface chemistries. Cells were cultured in serum and serum-free environments in order to distinguish the effects of adsorbed serum proteins on cell adhesion. Overall, comparing these cell types and biomaterials should provide a fundamental understanding of surface variables that control cell adhesion and therefore provide a knowledge base for future biomaterial design.

2. Basic laser spallation technique

In the laser spallation experiment developed to measure the tensile strength of a thin film interface, a 2.5 ns long Nd:YAG laser pulse is impinged over a 3 mm diameter area on a 0.5 μm thick Al film sandwiched between the back surface of a substrate disc (25 mm diameter and 1 mm thick) and a 50–100 μm thick layer of SiO_2 [14–28]. The melting-induced expansion of Al under confinement generates a compressive stress wave (with 1 ns rise time) directed towards the test coating, which is deposited on the substrate's front surface. The compression stress pulse reflects into a tensile wave from the coating's free surface and leads to its spallation (complete removal) at a sufficiently high amplitude. During this film separation process, the transient free surface velocity of the coating is continuously recorded using a state-of-the art optical

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