REAL-TIME MONITORING OF CELL-SURFACE INTERACTIONS

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INTRODUCTION

Cell adhesion, and the biological processes that depend on or are modulated by adhesion, has become a major avenue of investigation in nearly every field of biomedical research. Quantitative characterization of the physical interaction between cell and substrate is essential for the optimization of implant biomaterials and scaffolds for tissue engineering for which tissue integration is a primary determinant of performance. In this paper, a novel Acoustic Shear Wave Biosensor for the Analysis of Cell Adhesion and Structure (ABACAS) is presented.

The basic element of the ABACAS is a piezoelectric Acoustic Shear Wave (ASW) sensor. Exciting the sensor with an alternating voltage produces standing acoustic waves within the sensor, and the sensor behaves as a highly sensitive resonator, transmitting a shear wave into the medium [1]. The distribution of shear mechanical displacement generated by the transducer immersed in a Newtonian liquid is given by the classical solution due to Stokes (Figure 1). The penetration depth decreases with increasing frequency. However, an increase of viscosity can significantly extend the depth of penetration to tens of microns. We will make use of these phenomena to analyze the characteristic length scale of structures at the interface and the dynamically changing mechanical properties of adherent cells.

METHODS

Measurement System

The measurements were performed using a thickness shear mode (TSM) sensor operating the fundamental resonant frequency of 5 MHz and the odd harmonics of 15 and 25 MHz. The TSM frequency responses (S_{21} scattering parameter) were measured using a HP 8595 Network Analyzer based measurement system, which provided the resonant frequency and amplitude of the sensor as a function of time.

Microsphere Suspensions

Carboxylated polystyrene microspheres (1, 10 and 90 μm diameter; Polysciences, Warrington, PA) suspended in Dulbecco's Modified Eagles Media (DMEM; Gibco) were used. Suspensions were prepared by diluting the stock solution (2.7% g/ml) 1:5 with tissue culture medium DMEM. Final concentrations of spheres were $9.8x10^9$, $9.8x10^6$, and $13.5x10^3$ spheres/ml, respectively.

Endothelial Cell Culture

Bovine Aortic Endothelial Cells (BAECs) were isolated from calf ascending aorta as previously described [2]. For long-term experiments, Cells were plated onto the sensor surface, and the TSM chamber was kept in a humidified, 37° C incubator with 5% CO_2 in air. For short-term sedimentation, adhesion and spreading experiments, cell suspensions were added to the chamber connected to the analyzer and recording continuously. The volume of the suspension added was kept constant for all experiments so as not to influence the sedimentation time.

RESULTS AND DISCUSSION

Since the behavior and structure of cells at the interfaces is complex, we used a physical model system to characterize the sedimentation process and to evaluate the ability of the sensor to detect structural features of different characteristic length scales. The

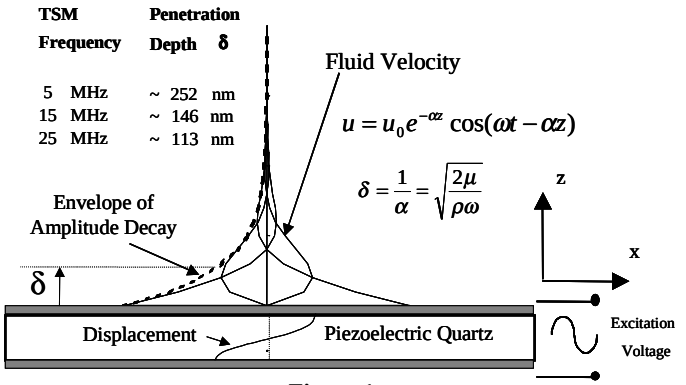

Figure 1.

sedimentation of polystyrene microspheres was monitored as a function of time using three different operating frequencies of the TSM sensor. Accumulation of polystyrene spheres at the TSM surface causes changes in the resonant amplitude with a characteristic time constant that corresponds to the predicted time to fill a single layer of spheres on the surface (Figure 2A).

The relationship between the penetration depth and the characteristic length scale of the structure at the interface is demonstrated by monitoring the sedimentation process using three particle sizes with three frequencies and, therefore, three penetration depths. The sedimentation kinetics of the 1.0 μm particles is accurately detected at all three operating frequencies (not shown). In contrast, the 10 μm particles are only detected at the lower two frequencies corresponding to greater penetration depths. The 90 μm particles were not detected at any of the frequencies (not shown).

These experiments demonstrate that by probing the medium at different depths, we can sensitively monitor events or structural changes at the interface and distinguish between signals due to structures located at different distances from the sensor surface. We used the same approach to characterize the interaction of living endothelial cells with the sensor surface. The sedimentation of bovine BAECs was measured by placing a suspension of cells on the sensor and monitoring the signal at 5 MHz for 1 hour (Figure 2B). The kinetics of sedimentation were similar to the 10 μm polystyrene spheres, which are of a size and density (1.05 g/ml) similar to the

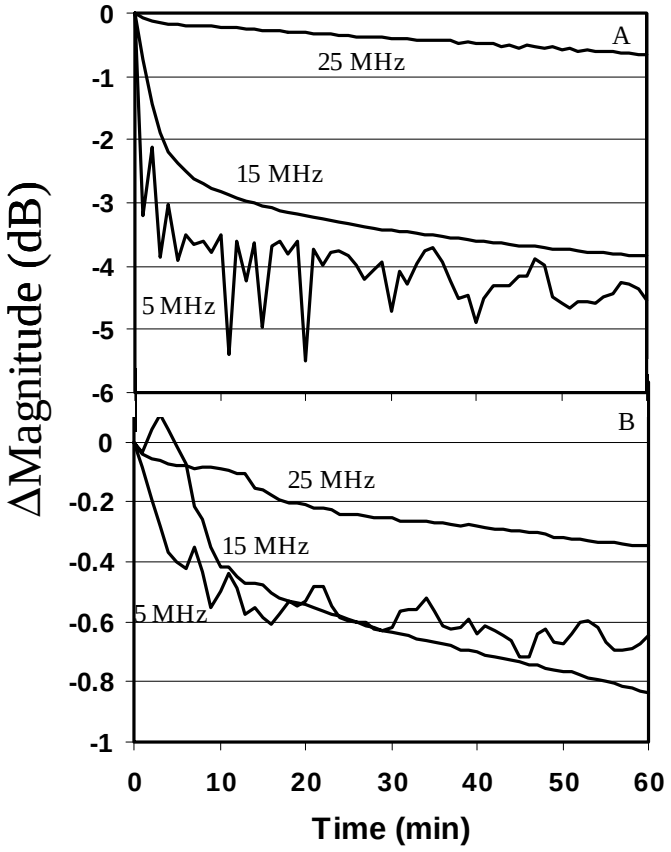

Figure 2.

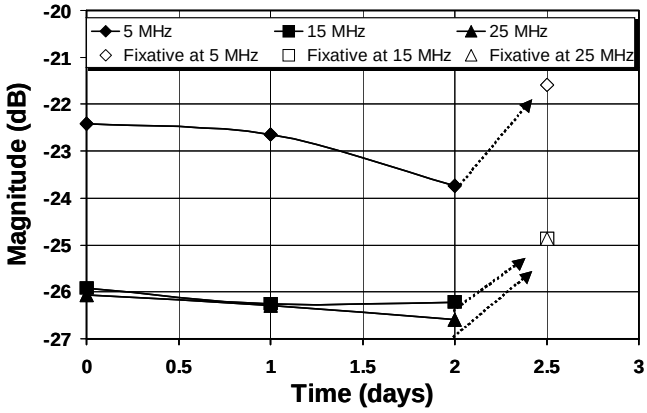

Figure 3.

endothelial cells. As with the 5 MHz microsphere experiment, the response to sedimentation alone (t < 10 minutes) produced only a small decrease in amplitude. However, the shorter penetration depths of 15 and 25 MHz excitation revealed evidence of an active biological process not present in the polystyrene sphere experiments. The decrease on the magnitude of the 15 MHz resonance peak resembled that for the microspheres, but with a delay of 5 minutes. Similarly, the 25 MHz signal dropped significantly at 15 minutes (Figure 4B). A decrease in magnitude indicates an increase in the viscous loss. The timing of the decrease in magnitude at successively shorter penetration depths may indicate sequential steps in the cell adhesion process including the establishment of adhesive bonding between the cells and the substratum (overcoming nonspecific repulsion) and, perhaps, the initial spreading of the cells.

To test the sensitivity of the sensor to the subtle changes in morphology with time in culture, BAECs were plated on the sensor at a slightly subconfluent density and monitored for two days. After reaching confluence, BAECs tend to lose their actin stress fibers in favor of a cortical arrangement that appears as a peripheral band along the cell junctions. The decrease in the 5 MHz resonance peak magnitude may represent this loss of structure in the region of the cell adjacent to the substrate (Figure 3). To verify that this technique indeed reports mechanical properties, the cells were fixed with formaldehyde. The crosslinking of cellular proteins causes the cell to stiffen and to behave more elastically with less viscous loss [4]. The increase in magnitude of the resonance peak is consistent with this interpretation.

ACKNOWLEDGMENTS

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REFERENCES

1. Ballantine, D. S., " Acoustic Wave Sensors", Academic Press., 1997.
2. Barbee, K. A., Davies, P. F., Lal, R. Shear stress-induced reorganization of the surface topography of living endothelial cells imaged by atomic force microscopy. Circ Res. 1994 Jan;74(1):163-71.
3. Barbee, K. A. Changes in Surface Topography in Endothelial Monolayers with Time at Confluence: Influence on Subcellular Shear Stress Distribution Due to Flow. Biochemistry and Cell Biology 73: 501-505 (1995).
4. Shroff, S. G., Saner, D. R. Lal, R. Dynamic micromechanical properties of cultured rat atrial myocytes measured by atomic force microscopy. Am.J.Physiol 269(1 Pt 1): C286-C292 (1995).