33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Real-time, *In vivo* Investigation of Mechanical Stimulus on Cells with Remotely Activated, Vibrational Magnetoelastic Layers

Hallie Holmes, Ee Lim Tan, Keat Ghee Ong, *Member, IEEE*, and Rupak M. Rajachar

***Abstract*—A system was developed for real-time, *in vivo* investigation of the relationship between local cell-level nano-mechanical perturbation and cell response to chemical-physical biomaterial surface properties. The system consisted of a magnetoelastic (ME) layer that could be remotely set to vibrate, at submicron levels, at a predetermined amplitude and profile. Experiments result indicated that submicron localized vibrations coupled with tailored biomaterial surface properties could selectively control cellular adhesion and possibly guide phenotypic gene expression. Practical application of this system includes modulation and monitoring of the surface of implantable biomaterials. The ME based vibrational system is the first of its kind for use *in vitro* for culture based mechanical testing, which could be readily deployed *in situ* as an *in vivo* system to apply local mechanical loads. It could be applied to specific implant surface sites and then subsequently sealed prior to long-term implantation. The potential advantage of this system over other similar approaches is that the system is translatable - the functional layer can serve as a "cellular workbench" material but could also be adapted and applied to the surface of implantable biomaterials and devices.**

I. Introduction

The control of interfacial phenomena is a significant area of research interest in biomaterials and is a key concern to the long-term success of many implanted biomaterials (healing response). The host response after biomaterial implantation essentially consists of an acute regenerative phase followed by a chronic fibrotic phase [1]. The extent and outcome of these phases is dependent on the physical and chemical properties of the implanted material as well as the local environment (cell and matrix character of the host tissue) post-implantation. Many implanted devices in biomedical engineering would benefit if effective control over molecular and cellular interactions at material surfaces could be achieved; e.g. modulation of protein adsorption; controlling the adhesive-behavior and activity of cells involved in the host response (fibroblasts, macrophages, giant cells) [2]. To this end a great deal of work has gone into understanding the properties governing protein and cell interactions at material interfaces including thermodynamic effectors such as electrostatic interactions and hydrophobicity; physical characteristics such as roughness and stiffness; and ligand-receptor interactions [3]. All of these factors impact cell adhesion, synthesis, and migration behavior that contributes to the staging of the host response and ultimate resolution of implanted biomaterials.

Cell adhesion on biomedical devices is a fundamental factor in integration after implantation. Implant materials have to be optimized regarding their surface characteristics such as chemistry, topography, and morphology. The effect of surface chemistry on adherent cell behavior has been a key area of research and defining these relationships will aid in establishing criteria for designing application specific biomaterials. Complexities in this process arise since there is not an integrated understanding of the means by which adherent cells interact with a surface. The concept that minimizing stable cell adhesion reduces cellular activity on a biomaterial surface has been an accepted tenet prompting numerous studies to investigate ways to modulate the adhesion process [3, 4].

In addition to inherent surface properties of materials, mechanical forces play a role in controlling the integrity and functionality of cells and tissues [4]. External forces are sensed by cells and translated into signals that induce cellular polarization. Cells convert energy from one form to another and respond to external environments by continually altering their structure. Short-term and long-term stimuli contribute cooperatively to the time-dependent activity level of a cell. Many normal and diseased conditions of cells are dependent upon their mechanical environment, and the deformation characteristics of cells can provide important information about their function. The deformability of cells is determined largely by the cytoskeleton, whose rigidity is influenced by both the chemical and mechanical environment of the cell including cell-cell and cell-matrix interactions. Cyclic stretching of cells is commonly used to mimic external forces exerted in the body. In such experiments, cells, adherent to an elastic substrate, orient with their long axis perpendicular to the stretch direction [5]. Such polarization events require a dramatic reorganization of cell-extracellular matrix contacts. The direct communication between cells and extracellular matrix is mediated by integrins that are associated with the actin cytoskeleton via a multi-protein complex of regulatory molecules. Such focal adhesion (FA) sites are thought to be crucial in the process of mechano-transduction and are involved in the transformation of mechanical into biochemical signals [6]. Ultimately, mechanical cues are a crucial factor in the maintenance of proper tissue specific cellular identity (mechanical set-point). A large body of evidence suggests that when the cellular microenvironment is disturbed as in tissue injury or repair many cell types are activated by non-physiologic (disturbed) mechanical stress

Manuscript received March 22, 2011.

Halley Holmes is with the Michigan Technological University, Houghton, MI 49931 USA (e-mail: eltan@mtu.edu).

Keat Ghee Ong is with the Michigan Technological University, Houghton, MI 49931 USA (ph: 906-487-2749; e-mail: kgong@mtu.edu).

Rupak M. Rajachar is with the Michigan Technological University, Houghton, MI 49931 USA (ph: 906-487-1129; e-mail: rupakr@mtu.edu).

and cytokines to take on a myofibroblastic phenotype. The result of this regained plasticity can have significant detrimental clinical consequences including the dramatic loss of function (tissue compliance) associated with contraction of burn associated scar tissue; loss of functionality of flexible or permissive membrane-based biosensors; and restenosis associated with percutaneous cardiovascular interventions such as stents.

Generating "switchable" materials surfaces that are externally controllable has become an increasingly important goal in the area of biomaterials. Optimal therapeutic approaches to the treatment of tissue injury (implantation) should be minimally invasive and create a stable implant-tissue interface. The advantages to this are improved patient comfort, reduced risk of infection, and improved stability and service-life of implanted devices. The system described in this work has the potential to introduce a novel real-time "self aware and post-deployment activated" approach to the design of implant surface modification, mitigation of the host response.

Traditional approaches to control the tissue-biomaterial interface aim to minimize fibrous tissue formation by targeting myofibroblasts using anti-fibrotic drugs and biomaterial surface modification. The former look to modulate the interface through controlling inflammation, cell activity, and angiogenesis; and tend to only be effective for short periods of time. The later have focused on biomaterial treatments that vary surface chemistry to promote favorable integration outcomes and to minimize the fibrotic response post-implantation. Although these transient methods have indeed improved stable tissue integration, no surface modification has yet been developed that can actively monitor and modulate the material surface at a soft tissue-biomaterial interface in-situ. As such, the long-term success of most implantable biomaterials remains a major issue. For this reason, a vibration layer was developed to modulate local cell adhesion and activity at the tissue-implant interface. The vibration layer is based upon a ME material that, when subjected to an applied magnetic DC or AC field, can be remotely set to apply a static or dynamic (vibration) strain at a predetermined amplitude and frequency [7]. When placed inside a magnetic field, the ME material changes dimensions converting magnetic energy into mechanical energy, as illustrated in Fig. 1.

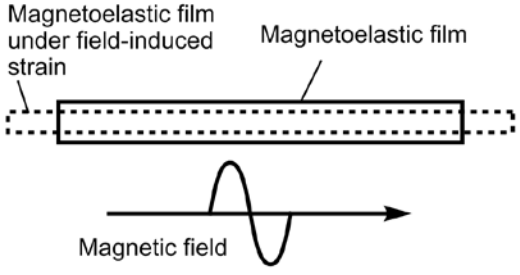

Fig. 1. Illustration of ME material physical response to an applied magnetic field. An active AC magnetic field produces an oscillating displacement (δ < 1 μm) parallel to the ME material.

Previous investigations have utilized ME materials as biosensors to measure a wide range of biological quantities including coagulation, pressure, fluid flow and viscosity, and recently cancer cell growth [8]. This work, however, is to employ sub-micron mechanical strains produced by the ME material as a therapeutic tool to control cell adhesion and myofibroblastic activity (Fig. 2).

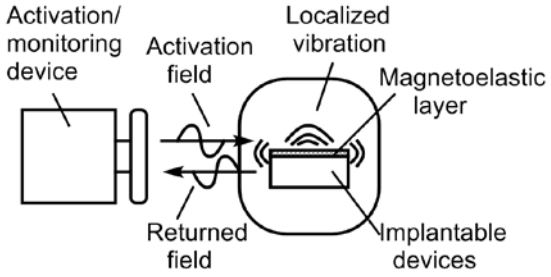

Fig. 2. Schematic of active vibration-coating system. Use of an applied external magnetic field can be used to monitor and control material surface *in situ.*

The novelty of this technology is that instead of only transient chemical or physical control at the interface, it relies also on a physically attenuating barrier, where small local vibrations can be used to selectively modulate cell adhesion to minimize fibrosis and promote proper integration at a soft tissue-implant interface. Since the amplitude and profile of the vibration can be easily controlled with a changing magnetic field, it would allow for a need-dependent treatment approach for the service life of an implant. The coating vibration can also be monitored and controlled *in vivo* at the site of implantation, via the induced magnetic field and a changing excitation field (see Fig. 2). Most approaches to date do not allow for modulation of the implant characteristics once deployed *in vivo*. With this technology, it may be possible to monitor implant behavior as well as affect changes in the local mechanical and chemical environment. In addition, this technology will further impact the understanding of local vibrations as a therapeutic tool. Only recently has the mechanism of larger amplitude and frequency vibration (ultrasound) therapeutic effects on cell behavior become clearer. The site-specific approach may also lead to new strategies and applications for the use of these platform materials in implantable biomaterials, similar to how focused ultrasound beams can be used to modulate delivery vehicles and tissue structure to offer the opportunity for local drug and gene delivery. The work described here looks to establish the basic character for an ME platform that can 1) function long-term in-situ and 2) retain is vibration character for actuating cell specific behavior.

II. Experiments

The ME materials need to remain stable and function for long periods of time in contact with an aqueous environment in culture and *in vivo*. Conformational Parylene-C was chosen as the base coating for these experiments because it is the established standard for coating implantable devices

including pacemakers. Adding a stiff conformational coating, however, can have significant effects on vibration character (dampening). Therefore, experiments were performed to determine the effect of Parylene coating on the vibration frequency and amplitude.

Metglas 2826MB ($Fe_{40}Ni_{38}Mo_4B_{18}$), purchased from Metglas, Inc., was selected as the magnetoelastic layer. The as-purchased Metglas material was sheared into 12.7 mm × 5 mm × 28 μm strips. Conformal Parylene-C layers were coated on these strips using a SCS Parylene coating system, with thickness controlled by coating time. The ME resonances of these strips were then monitored with a customized detection system, and their weights were measured with a microbalance. The variations in resonant frequencies and amplitudes were compared to the weight increase to establish a relationship among them.

The Parylene-coated ME materials were not inherently cell adhesive and thus the surface of these materials was spin-coated with chitosan to promote uniform cell adhesion. Coating weight was assessed using the method described above. *In vitro* toxicity tests were conducted to examine the biocompatibility of each surface treatment. Specifically, the coated samples were tested in culture with fibroblasts (ATCC-L929). The cells were seeded directly onto substrates and incubated at 37°C and 5% CO_2 for one week. A single media change was made at 3 days. Quantitative cell survival was assessed using a live/dead fluorescence assay and validated using a trypan blue exclusion method.

To assess cell morphological response towards mechanical stimuli, cells were seeded onto the ME layers functionalized with chitosan at a density of 2×10^4 cells/cm^2 and incubated at 37°C with 5% CO_2 for 48 hours. Vibrations were then applied for 1 hour. Cells were fixed with 4% paraformeldehyde and dehydrated with ethanol. Images of adherent cells on each sample were taken using a JOEL JSM-6400 SEM and morphological measurements were made using Bioquant analysis. Parameters of interest for this work were cell area and shape factor. A shape factor array measures the ratio of an object area to the area of a circle with an identical perimeter. Effectively, this is a measurement of an object's "roundness" on a scale from 0 (straight line) to 1 (perfect circle).

III. Results and Discussion

Vapor deposition with Parylene restored cell viability to levels of controls (Fig. 3) for long-term testing while maintaining the vibration character of the ME layer. Fig. 4 plots the resonance behaviors of the layer with 5 μm, 10 μm, and 20 μm coating of vapor deposited Parylene.

As mass loading increased, the resonant frequency of the sensor was expected to decrease due to the increasing damping effect. The resonant frequency variation of the sensor due to mass increase from a uniform coating can be determined with [9]:

$$\frac{f_c}{f_0}=\sqrt{\frac{1+\alpha\left(E_c/E_s-1\right)}{1+\alpha\left(\rho_c/\rho_s-1\right)}} \tag{1}$$

where E_c and E_s are the elasticity of the coating and sensor, respectively, ρ_c and ρ_s are the density of the coating and sensor, respectively, f_c is the resonant frequency of the coated sensor, and f_0 is the resonant frequency of the uncoated sensor. The coefficient α can be determined as:

$$\alpha=\frac{m_c/m_s-1}{m_c/m_s-1+\rho_c/\rho_s} \tag{2}$$

where m_c is the total mass of the sensor and coating, and m_s is the mass of the uncoated sensor.

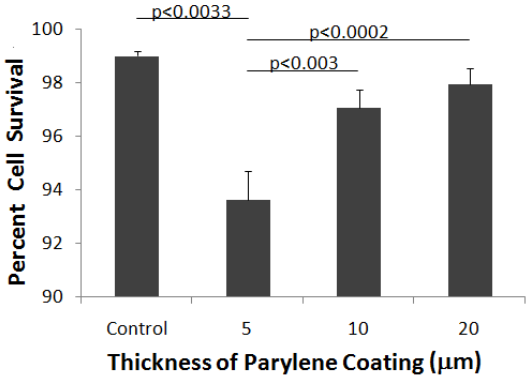

Fig. 3. Vapor deposition with Parylene restores cell viability for long-term testing while maintaining ME vibration character. Vibrational sweeps and masses were taken to establish the initial character of ME sensors. The ME sensors were then treated with 5 μm, 10 μm, and 20 μm coating of vapor deposited Parylene. Sensors were then incubated for 14 days in Dulbecco's Modified Eagle's Medium containing fetal bovine serum at 37° C. Indirect cytotoxicity tests were then taken to determine cell viability.

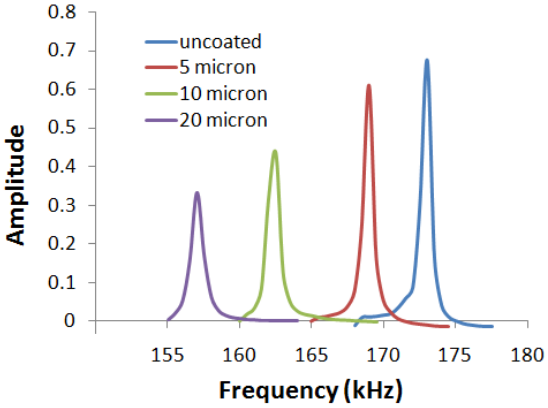

Fig. 4. The resonance behavior of an uncoated ME sensor and sensors treated with 5 μm, 10 μm, and 20 μm coating of vapor deposited Parylene.

Fig. 5 plots the change in resonant frequency as a function of mass loading. The expected resonant frequency change was also calculated with Eq. (1) and plotted. The parameters used for the calculations were obtained from the manufactures and are E_c = 3.2 GPa, E_s = 100 GPa, ρ_c = 1.289 g/cm^3 and ρ_s = 7.9 g/cm^3. The measured and predicted resonant frequencies changes show good agreement.

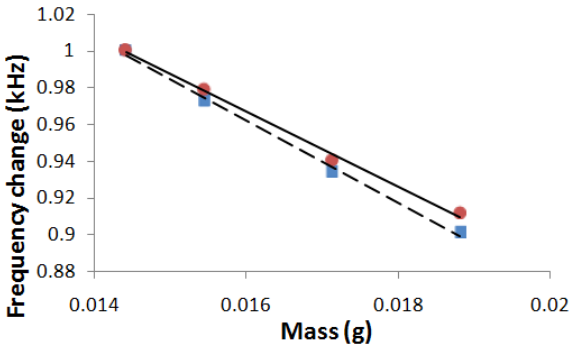

Fig. 5. The resonant frequency of a ME sensor decreased linearly with increased Parylene coating weight. Red dots are the measured resonant frequency change (f_c/f_0), and blue squares are the predicted change calculated with Eq. (1).

TABLE I
ME VIBRATIONS (1HR, 170-176 KHZ) MODULATE CELL MORPHOLOGY.

Morphological Parameter	Controls (V-)	Treatment Group (V+)
Normalized Cell Area	1.00*	0.683*
Shape Factor	0.529#	0.763#

[* AND # INDICATE SIGNIFICANT DIFFERENCES ($p<0.05$)]

Quantitative cell survival data showed no significant difference in cell survival between vibrated and non-vibrated samples with all measures showing greater than 95% survival [10]. A subsequent experiment was conducted to examine the effect of the vibration profile (amplitude and period between vibrations) on cell attachment. These results indicated that cell attachment responded in a dose dependent fashion to changes in signal amplitude and frequency (data not shown). More importantly Table I shows that vibration loading can significantly affect morphological character of adherent cells (L929 fibroblasts). Modulation of morphological character using surface modification approaches has been shown to affect cell behavior including survival, differentiation, and migration. These results suggest that sub-cellular surface strains may be capable of the same results with the benefit of in-situ modulation.

IV. CONCLUSION

This work establishes a platform sub-micron loading system based on a Parylene coated ME substrate. We show these materials can be prepared for use *in situ* without loss of mechanical character (i.e. the capacity to modulate cell behavior). To our knowledge this loading system is the first of its kind, one that can be used for *in vitro* and *in situ* application of local mechanical loads.

REFERENCES

[1] T. A. Wynn, "Cellular and molecular mechanisms of fibrosis," *The Journal of pathology,* vol. 214, 2008, pp. 199-210.

[2] M. J. A. Van Luyn, M. Harmsen, D. Luttikhuizen, "Cellular and molecular dynamics in the foreign body reaction," *Tissue Engineering,* vol. 12, 2006, pp. 1955-70.

[3] X Liu, J. Lim, H. Donahue, R. Dhurjati, A. Mastro, E. Vogler. "Influence of substratum surface chemistry-energy and topography on the hFOB cells: Phenotypic and genotypic responses observed *in vitro*." Biomaterials, vol. 28, 2007, pp. 4535-4550.

[4] G. Khang, S. Lee, J. Lee, Y. Kim, H. Lee. "Interaction of fibroblast cells on PLGA surface with wettability chemogradient." *Bio-Medical Materials and Engineering*, vol. 9(3), 1999, pp. 179-187.

[5] C. Chen, J. Tan, J. Tien. "Mechanotransduction at cell-matrix and cell-cell contacts." *Ann Rev Biomed Eng*., vol. 6, 2004, pp. 275-302.

[6] B. Geiger, J.P. Spatz, A. Bershadsky. "Environmental sensing through focal adhesions." *Nature Rev Mol Cell Biol.*, vol. 10, 2009, pp. 21-33.

[7] C. A. Grimes, C. S. Mungle, K. Zeng, M. K. Jain, W. R. Dreschel, M. Paulose, K. G. Ong, "Wireless magnetoelastic resonance sensors: a critical review," *Sensors*, vol. 2, p2002, pp. 294-313.

[8] K. G. Ong, M. Zourob, R. Shao, B. Horton, B. D. Pereles, and E. L. Tan, "Implantable Biosensors for Real-time Strain and Pressure Monitoring," *Sensors,* vol. 8, 2008, pp. 6396-6406.

[9] C. A. Grimes, C. S. Mungle, K. Zeng, M. K. Jain, W. R. Dreschel, M. Paulose, K. G. Ong, "Wireless magnetoelastic resonance sensors: a critical review," *Sensors*, vol. 2, 2002, pp. 294-313.

[10] E. Vlaisavljevich, L. Janka, K.G. Ong, R.M. Rajachar, "Magnetoelastic Materials as Novel Bioactive Coatings for Control of Cell Adhesion," *Biomedical Engineering, IEEE Transactions on*, vol. 58, 2011, pp. 698-704.