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Control of cellular adhesion and myofibroblastic character with sub-micrometer magnetoelastic vibrations

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ABSTRACT

The effect of sub-cellular mechanical loads on the behavior of fibroblasts was investigated using magnetoelastic (ME) materials, a type of material that produces mechanical vibrations when exposed to an external magnetic AC field. The integration of this functionality into implant surfaces could mitigate excessive fibrotic responses to many biomedical devices. By changing the profiles of the AC magnetic field, the amplitude, duration, and period of the applied vibrations was altered to understand the effect of each parameter on cell behavior. Results indicate fibroblast adhesion depends on the magnitude and total number of applied vibrations, and reductions in proliferative activity, cell spreading, and the expression of myofibroblastic markers occur in response to the vibrations induced by the ME materials. These findings suggest that the subcellular amplitude mechanical loads produced by ME materials could potentially remotely modulate myofibroblastic activity and limit undesirable fibrotic development.

1. Introduction

The ability to repair damaged tissues by replacing lost cells is one of the paramount functions of living organisms. However, after sustaining damage to tissues, such as that caused by biomedical implants, the wound healing response may enter a phase of fibroplasia or fibrosis. This process can become pathogenic if it proceeds without control. Excessive fibrosis results from uncontrolled myofibroblastic activity, particularly the production and significant remodeling of extracellular matrix components (ECM) (Wynn, 2007). Myofibroblasts, derived from local mesenchymal cells (e.g. fibroblasts), are essential to the wound healing process, as they produce ECM components to promote wound contraction. This process allows epithelial or endothelial cells to reach the site of injury and repair damaged tissue (Wynn, 2007, 2008). When a persistent irritant, such as an implant, is present, the growth factors and cytokines that promote myofibroblastic activity are continually expressed, resulting in the overproduction of ECM components (Wynn, 2007). The excessive accumulation of these components at the soft tissue-implant interface can lead to the destruction of healthy tissue architecture and the mechanical instability of an implant, which presents a crucial impediment to the long-term success of many implantable devices (Wynn, 2007, 2008; Ziats et al., 1988).

Current treatments for reducing implant fibrosis include pharmaceutical options and pre-implantation design. Anti-fibrotic drugs attempt to alleviate the effects of fibrosis by controlling inflammation, myofibroblastic activity, and angiogenesis (Wynn, 2007, 2008), whereas biomaterial surface modifications focus on altering surface chemistry with biomaterial coatings that diminish the fibrotic response post-implantation (Anderson et al., 2008; Ziats et al., 1988). While these methods have attenuated the fibrotic response and have remarkably improved stable tissue integration, there is still no treatment process that ultimately prevents fibrous marsupialization of the soft tissue-implant interface (Anderson et al., 2008; Luttikhuizen et al., 2006; Lydon et al., 1985; Pendegrass et al., 2006; Ziats et al., 1988).

In this work, we explore the potential of a non-invasive method to remotely control cellular adhesion and behavior at the soft tissue-implant interface. The underlining principle of this study is based upon the innate properties of magnetoelastic (ME) materials. ME materials are magnetostrictive, which allows them to convert magnetic energy into mechanical energy and *vice versa* (Grimes et al., 2002). As a result, these materials change shape in response to a magnetic field. These changes are in the form of a uniaxial deformation and occur in response to DC and AC magnetic fields, resulting in static or dynamic (vibration) strains (Grimes et al., 2002, 2011). The deformation seen by the ME material is a function of the initial length of the material and the strength of

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the applied magnetic field (Grimes et al., 2002, 2011), therefore the magnitude of this deformation can be controlled through prefabrication design of the ME material and the parameters of the applied magnetic field. However, the maximum possible deformation of ME materials is still on the order of sub-micrometers (submicron) (Grimes et al., 2002, 2011), which makes these materials good candidates for *in situ* use because their functionality does not present a physical danger to surrounding soft tissue. Additionally, the movement of these materials generates a magnetic field that can be remotely detected (Cai et al., 2000, 2001; Cai and Grimes, 2000; Grimes et al., 1999, 2000, 2002, 2011; Grimes and Kouzoudis, 2000; Jain et al., 2000, 2001; Kouzoudis and Grimes, 2000; Loiselle and Grimes, 2000; Stoyanov and Grimes, 2000). This secondary magnetic field has been shown to be dependent on the mass applied to the surface of the ME material and the stiffness (Young's modulus) of the surrounding microenvironment (Grimes et al., 2002, 2011). For these reasons, ME materials have already seen use in a variety of biomedical applications by functioning as physical sensors for temperature (Jain et al., 2000, 2001), pressure (Grimes et al., 1999; Jain et al., 2001; Kouzoudis and Grimes, 2000), liquid viscosity and flow velocity (Grimes et al., 2000; Grimes and Kouzoudis, 2000; Loiselle and Grimes, 2000; Stoyanov and Grimes, 2000), and as chemical sensors for carbon dioxide, pH, and ammonia (Cai et al., 2000, p. 2, 2001; Cai and Grimes, 2000).

Previous work has shown that sub-micron vibrations induced by the ME materials (for simplicity, we refer to them as the ME vibrations) are capable of preventing cell adhesion *in vitro* without inducing apoptosis or cellular necrosis (Vlaisavljevich et al., 2011), and that an ME material can be functionalized for long-term use *in vivo* without sacrificing its magnetostrictive functionality (Holmes et al., 2012; Vlaisavljevich et al., 2013). This work expands upon these findings by investigating the cellular mechanisms dictating the observed responses to ME vibrational loading. We hypothesize ME vibrations direct cell behavior through matrix adhesion contacts (focal adhesion complexes) that connect cells to the ME substrate (Chiquet et al., 2007, 2009). This process is known as mechanotransduction and occurs when mechanical loads create stresses on sites of focal adhesions. These stresses are conducted through the cell membrane or cytoskeleton and initiate signaling cascades that can induce changes in cell morphology, adhesion, or gene expression (Chiquet et al., 2003, 2007, 2009; Lambert et al., 1998; Wang et al., 1993). This process is essential for maintaining connective tissue homeostasis, as tissues must constantly adapt to a dynamic range of physiologic loads (Kjaer, 2004). Mechanotransduction has been investigated through many methods, such as axial stretching of elastic membranes (Wang et al., 2007) or imposing fluid shear stress on cell cultures (Chen et al., 1999). However, this process is not fully understood, and relatively little is known about the effects of sub-cellular mechanical loads on cell behavior. Therefore, we sought to accomplish three primary objectives in this work: 1) characterize how physical properties (mechanical parameters of applied vibrations) affect cell adhesion, 2) characterize the effect of ME vibrations on cell proliferation and morphology, and 3) determine if ME vibrations can influence myofibroblastic character.

2. Materials and methods

The magnetoelastic material, Metglas 2826 MB ($Fe_{40}Ni_{38}Mo_4B_{18}$) a commercial magnetoelastic film purchased from Metglas Inc. was used for all experiments. The material (thickness = 26 μm) was mechanically sheared into strips (5 mm × 12.7 mm) and annealed for 2 h at 125 °C to reduce the internal stress of the material. Annealed ME strips were coated with Parylene-C using a PDS 2010 LABCOTER™ 2 Parylene deposition system. Parylene-C coated ME materials were functionalized for cell adhesion by etching the Parylene-C surface for 30 s with O_2 plasma.

L929 fibroblasts (ATCC) were used for all experiments. Cells were maintained in culture medium composed of Dulbecco's Modified Eagles Medium D5796 supplemented with 10% FBS and 0.5% Penicillin-Streptomycin (ATCC). Cells were washed in PBS (pH 7.4) and trypsinized (0.05% Trypsin/0.53 mM EDTA in HBSS; Cellgro) to induce cell detachment for experimental seeding. Live cell staining was performed using Calcein-AM (Invitrogen) and all imaging was performed on an Olympus BX51 upright microscope unless otherwise stated. Preliminary experiments established that cells (seeded at 1×10^4 cells/cm^2) became adherent after approximately 6 h and reached 70–80% confluence after approximately 48 h at 37 °C and 5% CO_2 on plasma etched Parylene-C coated ME substrates.

To stimulate cells with sub-micron vibrational loads, ME materials were activated using the ME vibrational loading system previously developed (Vlaisavljevich et al., 2011). Control vibration negative groups (cells cultured on ME materials that were not magnetically activated) were seeded and imaged concurrently with experimental groups. Cellular adhesion data is presented as the percentage of remaining adherent cells in comparison to non-vibrated controls, unless otherwise indicated. The ME substrates, having a vibration resonant frequency of approximately 165 kHz (Holmes et al., 2012) were activated by an AC magnetic field (sweeping frequencies of 160–170 kHz with a step of 500 Hz).

All experiments were performed in triplicate unless otherwise stated, and all statistical analyses were performed using student's *t*-test on JMP software; p-values less than 0.05 ($p < 0.05$) were considered significant.

2.1. Load profile

The magnetic field generated by the *in vitro* loading system was tuned to produce the maximum strain of ME materials (12 ppm – a limit predetermined by innate material properties (Grimes et al., 2002)). For the ME materials used in this study, this strain provides an approximate displacement of 0.15 μm, where displacement is defined as the product of strain and initial length (12.7 mm) of the ME materials.

In order to load cells with vibrations of lower magnitude, the frequency range of the applied magnetic field was adjusted to provide a vibrational amplitude two-thirds (2/3) of maximum magnitude, as further frequency shifts would substantially diminish the vibration amplitude. The change in vibration amplitude was verified in real time by monitoring the change in the secondary magnetic field response of the ME material. This magnitude of vibration provided an approximate displacement of 0.10 μm. ME vibrations of each magnitude were applied to cells for 1 h following 48 h of incubation. Vibrations of 0.15 μm were also applied for 30 min (half of a typical loading duration) to determine if cell response was dependent on loading time. The interval between each loading cycle was adjusted through the controlling software for the ME vibrational loading system. Cells were loaded with ME vibrations for 1 h with 1 s or 10 s delays between each applied vibration. A 1 s delay is the minimum time required for the control system to activate, interrogate, and record data from ME materials (Vlaisavljevich et al., 2011), and a 10 s delay provided an order of magnitude change to understand how this recover time affects the cellular response.

2.2. Proliferation

ME vibrations were applied to cells cultured on plasma etched Parylene-C ME substrates after 6 or 48 h of incubation. Live stain-