31st Annual International Conference of the IEEE EMBS
Minneapolis, Minnesota, USA, September 2-6, 2009

Controlling Cellular Biomechanics of Human Mesenchymal Stem Cells

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Abstract — **The therapeutic efficacy of human mesenchymal stem cells (hMSCs) depends on proper characterization and control of their unique biological, mechanical and physicochemical properties. For example, cellular biomechanics and environmental mechanical cues have been shown to critically influence cell commitment to a particular lineage. We characterized biomechanical properties of hMSCs including cytoskeleton elasticity and plasma membrane/cytoskeleton coupling. As expected, during osteogenic differentiation of hMSCs, the cellular biomechanics is remodeled, and such remodeling precedes up-regulation of the osteogenic markers. Further, application of an electrical stimulation modulates the cellular biomechanics and therefore may be used to facilitate stem cell differentiation for stem cell-based tissue engineering.**

I. Introduction

Biomechanics is known to play an important role in cell metabolism [1]. Cell phenotype, tissue-specific functions, and fate critically depend on extracellular mechanical environment. In different tissues the cells should adapt their mechanical properties to those of the extracellular matrix, and properly respond to numerous environmental mechanical cues. The mechanical properties, such as the cytoskeleton elasticity, membrane tension, adhesion strength may play an important role in cell homeostasis and differentiation [2],[3]. For example, the pluripotent bone marrow-derived human mesenchymal stem cells (hMSC) can be differentiated into many tissue-specific lineages, including osteoblasts, chondroblasts, adipocytes, and others [4],[5]. However, the complexity of events associated with transformation of these precursor cells leaves many unanswered questions about morphological, structural, proteomic, and functional changes in stem cells. The knowledge of hMSC behavior would allow more effective approaches to cell expansion in vitro, and regulation of their commitment to a specific phenotype. It has been shown recently that the promising differentiation potential of these cells can be realized not only by biochemical induction factors, but also by a physical stimulation including substrate stiffness and electrical stimulation [6],[7].

The cells of mesodermal origin show a wide spectrum of mechanical properties. This suggests that cellular biomechanical properties may be significantly altered during stem cell differentiation, and an external physical manipulation of cellular mechanics could be used to enhance cell differentiation efficacy. The control of cell behaviors might be feasible through manipulation of the cellular properties using various external physical stimuli, including electric fields. Indeed, external electric field has been shown to induce a variety of cellular and molecular responses including, to name just a few, microfilament reorganization, cell surface receptor redistribution, changes in intracellular calcium dynamics, galvanotropic cell migration and orientation, enhanced stem cell differentiation, and angiogenesis [8]-[12]. Moreover, electrotherapy has been successfully used clinically for bone fracture treatment, nerve fiber repair, soft tissue regeneration, and cancer chemotherapy [13],[14].

Elucidation of the electrocoupling mechanisms is expected to establish a rational paradigm for electrically assisted differentiation of stem cells into pre-selected phenotypic lineage. In this study, we examined and characterized the changing mechanical parameters of cell cytoskeleton and the plasma membrane during hMSC osteogenic differentiation, and analyzed the cellular mechanical responses to an external electrical stimulation. Careful characterization of electrically mediated mechanical responses is expected to lead to enhanced cell differentiation and other tissue engineering applications.

II. Materials and Methods

A. Cell culture, differentiation and electric field exposure

Human mesenchymal stem cells (Tulane Center for Gene Therapy, New Orleans, LA), and normal human osteoblasts (ATCC, Manassas, VA) were maintained in Dulbecco's modified Eagle's culture medium with 15% fetal bovine serum. Osteogenic induction medium contained 10 nM dexamethasone, 50 μM L-ascorbic acid, and 20 mM β-glycerophosphate. Cells were exposed to a physiologically relevant 2 V/cm dc electric field for 60 min in a custom-designed stimulation chamber that minimizes Joule heating and electrode byproducts. The corresponding control experiments were conducted by incubating the cells in the same experimental conditions without an active stimulus.

Manuscript received April 7, 2009. This work was supported in part by National Institute of Health grant EB006067 and by a grant from the Office of Navy Research (N00014-06-1-0100). The AFM used to measure the cellular mechanical properties was purchased with Defense University Research Instrument Program grant N00014-04-1-0805.

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B. *Microindentation elasticity test*

The live cell elasticity was measured with a Novascan atomic force microscope (Novascan, Ames, IA). A 10 μm-diameter glass bead glued onto soft silicone nitride cantilever (spring constant 0.12 N/m, Veeco, Santa Barbara, CA) was used to indent the cells grown on a coverglass. To obtain a force curve, the cantilever descended toward the cell at a velocity of ~2 μm/s until a trigger force of 3 nN was reached. Viscous dissipation of energy is minimal at this speed, and force measurements are dominated by the cell's elastic behavior. To minimize the effect of glass substrate on the cell elasticity measurements, we used an indentation depth up to 500 nm (~ 10-15% of the average cell height) for data analysis. The force-distance indentation curves from 30-40 cells of each type and experimental condition were collected and analyzed according to the Hertz model to determine local cell elastic modulus [15].

C. *Membrane tether extraction*

Polystyrene 0.5 μm diameter beads covalently coated with anti-CD29 antibody and tightly bound to the cell membrane were used as handles for membrane tether extraction as described earlier [16]. Beads were trapped with infrared laser (1064 nm, continuous wave, 0.5 W, SpectraPhysics, Mountain View, CA). The laser beam was focused on at the cell surface with a 100x oil immersion objective, and moved in the focus plane by a system of two confocal laterally translated lenses. The trapped bead was optically displaced from cell surface at a constant speed of 1.5 μm/s and a constant force ~3 pN to extract membrane tether. The tether growth was observed and recorded with a CCD camera until the bead escaped from the trap. The average tether length was determined by tracking 30-40 beads positions from ~20 cells.

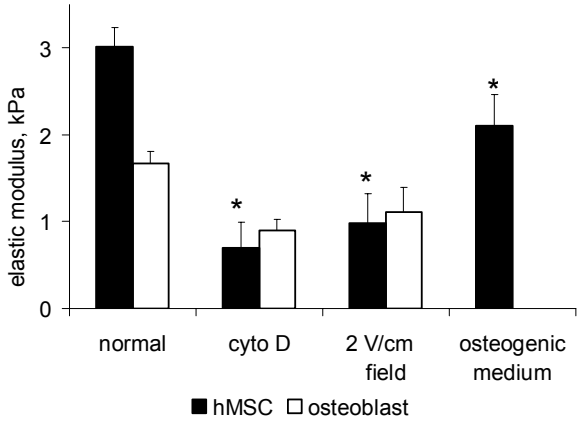

Fig. 1. Cell cytoskeleton elasticity measured with AFM. Elastic moduli of hMSCs and osteoblasts are reduced by cell treatment with cytochalasin D, electric field, or 10 day incubation in osteogenic medium. About 30-40 beads from ~20 cells per each condition were used. (*) significantly different from normal ($p<0.05$).

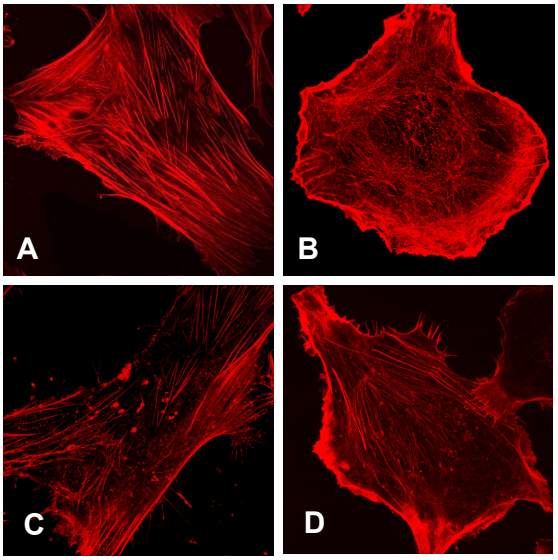

Fig. 2. Actin cytoskeleton organization. Thick actin stress fibers in normal hMSC (A), thin microfilament meshwork in mature osteoblasts (B). Actin remodeling in hMSC exposed to a 2 V/cm electric field (C), and in hMSC after 10 days in osteoinduction medium (D).

D. *Immunocytochemistry*

Fixed and permeabilized cells were immunostained with rhodamine-phalloidine for polymeric actin. Ezrin/radixin/moesin primary antibodies and secondary fluorescent antibodies were used for linker proteins staining. The samples were imaged with a laser scanning confocal system (BioRad, Hercules, CA).

III. Results and Discussion

The average elastic modulus 3.2 ± 1.4 kPa of hMSC is almost a 2-fold higher than 1.7 ± 1.0 kPa for osteoblasts (Fig. 1). Actin disassembly with 5 μM cytochalasin D decreased the elastic modulus to 0.7 ± 0.3 kPa in stem cells and to 0.9 ± 0.5 kPa in osteoblasts. However, microtubule disruption with nocodazole caused only an insignificant decrease in the cell elasticity in both cell types (data not shown). This result suggests that polymeric actin rather than microtubules provides the elasticity for cytoskeleton in these cells. Significant differences in the cellular elasticity of hMSCs and osteoblasts are likely due to different cytoskeleton organization in these cells. Indeed, while stem cells demonstrate a lot of thick actin stress fibers, extending throughout the cytoplasm (Fig. 2A), osteoblasts have fewer stress fibers and showed predominantly a thin dense meshwork of actins (Fig. 2B).

The average tether length in osteoblasts (4.0 ± 1.1 μm) is much lower than 10.6 ± 1.1 μm in undifferentiated stem cells (Fig. 3). The inhibition of actin polymerization with cytochalasin D resulted in almost a 2.5-fold tether length increase in osteoblasts, but had no effect on the tether length in hMSCs. As we postulated earlier, this result may be due to a weaker membrane-cytoskeleton coupling in hMSCs compared to fully differentiated cells [16]. Membrane is

physically attached to actin cytoskeleton at focal adhesion sites as well as by several specific linker proteins such as ezrin/radixin/moesin (ERM) family proteins. Two cell types in our study differ considerably in the membrane-cytoskeleton interaction. Thick actin stress fibers in stem cells provide a significant strength to cytoskeleton, but relatively few binding sites for ERM linkers. In contrast, a closely packed actin network in osteoblasts provides multiple binding sites for ERM proteins, as confirmed by immunofluorescent staining (Fig. 4A). Osteoblasts exhibit an overall stronger mechanical coupling between the membrane and cytoskeleton than hMSCs, as further evidenced by the tether extraction experiments (Fig. 4C).

During biochemically-induced hMSC differentiation into osteoblasts, their mechanics change correspondingly. Both the cytoskeleton elasticity and extracted membrane tether decrease to the values similar to those found in the mature osteoblasts after 10 days in the osteogenic medium (Figs. 1, 3). These mechanical changes are consistent with the replacement of thick actin stress fibers with a thinner actin network as osteogenic differentiation of hMSC progressed (Fig. 2D). However, the specific osteogenic markers such as mineralized calcium deposits and osteopontins are not seen until after ~ 2 weeks in the osteogenic media [4], [6]. Collectively, it suggests that remodeling of the cellular mechanics may precede hMSC osteogenic differentiation.

Cell biomechanical properties can be modulated by external physical stimulation. For example, cell exposure to a physiologically relevant 2 V/cm dc electric field for 60 min resulted in a significant decrease in the cytoskeleton elasticity of both stem cells and osteoblasts (Fig. 1). The cell elasticity decreases due to substantial actin cytoskeleton reorganization during exposure to an external dc electric field (Fig. 2C). Direct current and low frequency alternating current electric fields are unable to penetrate into the cell interior due to high resistivity of the cell membrane

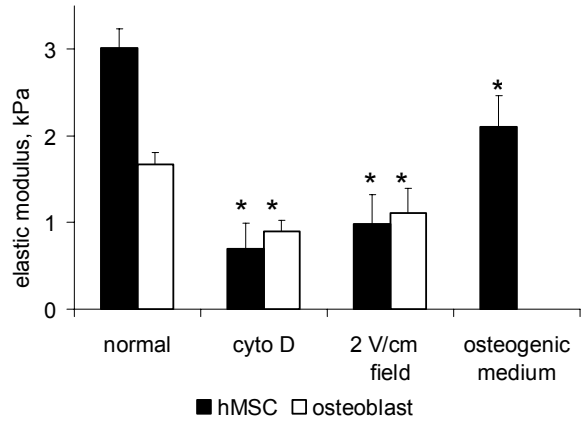

Fig. 3. Plasma membrane mechanics explored with LOT. Much longer tethers can be extracted from normal hMSC, than from mature osteoblasts or 10 day osteogenically-induced stem cells. Cytochalasin D and electrical stimulation increase tether length in osteoblasts, but not in hMSCs. About 300-500 force-curves from 30-40 cells per each condition were used. (*) significantly different from respective controls ($p<0.05$)

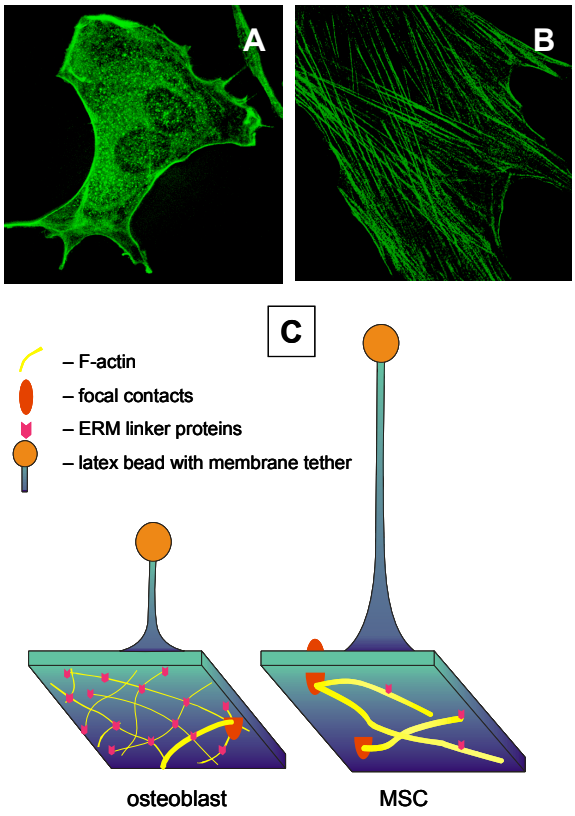

Fig. 4. ERM linker protein distribution in osteoblast (A) and hMSC (B). Experimental model of cell type-dependent membrane/cytoskeleton interaction (C). Stronger membrane/cytoskeleton adhesion in osteoblasts is mediated by more uniform actin-membrane linker distribution as compared to hMSCs.

(membrane conductivity is about 10^6 to 10^8 times smaller than that of the cytoplasm, [17]). As the direct electrocoupling to actins is excluded, molecular signaling pathways involved in the regulation of cell mechanics are likely initiated at the cell surface. Partial actin disassembly is likely to be attributed to an electrically induced increase in intracellular calcium concentration, which is mediated by a variety of well-characterized mechanisms [9],[10]. Besides, cell stimulation with an electric field caused membrane separation from cytoskeleton, as confirmed by an increase in extracted membrane tether length (Fig. 3). This effect was especially pronounced in osteoblasts, where initially the stronger membrane/cyoslekeleton coupling was considerably decreased by an electric field application. In contrast, in hMSCs this treatment did not cause any significant change in the membrane tether length likely due to originally weak membrane-cytoskeleton interaction in these cells. Interestingly, similar effects on the membrane mechanics are produced by ATP depletion, which leads to dephosphorylation and inhibition of ERM proteins. These small linker molecules can bind both to polymeric actins and integral transmembrane proteins only in their active phosphorylated state [18],[19]. Thus, an electrical stimulation might decrease the intracellular ATP level, inhibit ERM proteins, and separate the membrane from the cytoskeleton. Clearly, disruption of actin cytoskeleton itself

also results in the membrane dissociation from the cytoskeleton.

Periodic short-term application of a low intensity electrical stimulation has been shown to synergistically enhance the hMSC osteogenic differentiation [7]. Indeed, electrically induced disassembly of stress fibers in hMSCs can bring their cytoskeleton elastic and structural properties closer to those of fully differentiated osteoblasts. Cell recovery in the osteogenic medium after each a short-term electrical exposure (~ 30 min) could induce a further rearrangement of actins and ERM proteins into the osteogenic-type pattern, offering a plausible explanation for electrically facilitated hMSC osteogenic differentiation. Such a physical control of cell behaviors including differentiation and electromigration may have important implications for tissue engineering and regenerative medicine.

IV. Conclusion

The unique mechanical properties of human mesenchymal stem cells have been characterized using atomic force spectroscopy and laser optical tweezers. Biochemically induced osteogenic differentiation of hMSCs results in decreased cytoskeleton elasticity and increased membrane/cytoskeleton interaction that is typical for mature osteoblasts. These cellular mechanical parameters are also altered by cell exposure to a non-invasive electrical stimulation. The cell type-dependent biomechanical response to external electrical stimulation is mediated by actins and membrane/cytoskeleton linker proteins. Electrically-induced modulation of cell biomechanics may be used for control of cell differentiation and other cellular processes critical for tissue engineering. For example, as we have shown before, cell adhesion and orientation in 3D collagen matrix can be efficiently regulated by an electrical stimulus [11]. In addition, successful artificial tissue assembly from its component cells using dielectrophoresis and other electrokinetic techniques have also been reported [20]. These results are of particular interest for tissue engineering applications. Finally, we note that biomechanical regulation of stem cell differentiation can greatly minimize the number of growth factors that would otherwise be required for composite tissue engineering. Determination and appropriate use of combinations of the known physical and chemical cues (e.g., physicochemical cues) will undoubtedly facilitate the current research effort towards designing and engineering functional tissue constructs.

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