AIMS Bioengineering, 3 (4): 515-527.
DOI: 10.3934/bioeng.2016.4.515
Received: 04 October 2016
Accepted: 05 December 2016
Published: 09 December 2016

http://www.aimspress.com/journal/Bioengineering

Research article

Growth on elastic silicone substrate elicits a partial myogenic response in periodontal ligament derived stem cells

Daniel Pelaez [1,2], John H. Michel [1,2] and Herman S. Cheung [1,2,*]

[1] Geriatric Research, Education and Clinical Center (GRECC), Miami Veterans Affairs Medical Center, Miami, FL, USA
[2] Department of Biomedical Engineering, College of Engineering, University of Miami, Coral Gables, FL, USA

* **Correspondence:** Email: hcheung@med.miami.edu; Tel: +305-575-3388; +305-284-4466.

Abstract: The processes of cellular differentiation and phenotypic maintenance can be influenced by stimuli from a variety of different factors. One commonly overlooked factor is the mechanical properties of the growth substrate in which stem cells are maintained or differentiated down various lineages. Here we explored the effect that growth on an elastic silicone substrate had on the myogenic expression and cytoskeletal morphology of periodontal ligament derived stem cells. Cells were grown on either collagen I coated tissue culture polystyrene plates or collagen I coated elastic silicone membranes for a period of 4 days without further induction from soluble factors in the culture media. Following the 4-day growth, gene expression and immunohistochemical analysis for key cardiomyogenic markers was performed along with a morphological assessment of cytoskeletal organization. Results show that cells grown on the elastic substrate significantly upregulate key markers associated with contractile activity in muscle tissues. Namely, the myosin light chain polypeptides 2 and 7, as well as the myosin heavy chain polypeptide 7 genes underwent a statistically significant upregulation in the cells grown on elastic silicone membranes. Similarly, the cells on the softer elastic substrate stained positive for both sarcomeric actin and cardiac troponin t proteins following just 4 days of growth on the softer material. Cytoskeletal analysis showed that substrate stiffness had a marked effect on the organization and distribution of filamentous actin fibers within the cell body. Growth on silicone membranes produced flatter and shorter cellular morphologies with filamentous actin fibers projecting anisotropically throughout the cell body. These results demonstrate how crucial the mechanical properties of the growth substrate of cells can be on the ultimate cellular phenotype. These observations highlight the need to further optimize

differentiation protocols to enhance the application of cellular products in the fields of regenerative medicine and stem cell therapies.

Keywords: biophysical differentiation; cardiomyogenic induction; periodontal ligament derived stem cells; silicone; collagen

1. Introduction

In the field of stem cell therapy and regenerative medicine functionality of the resulting product is the ultimate goal. Functionality requires a fine tuned control of the phenotypic, genotypic as well as mechanical characteristics of engineered tissues. However, control of cellular phenotype, gene expression and ultimate lineage commitment engages complex mechanisms that can be influenced by a myriad of different factors. In such a multivariable system, overlooking one or several factors can drastically affect the outcome of a project and yield unremarkable results. It is thus necessary to consider as many factors as feasibly possible in the experimental design and execution stages of every study. While extensive research has been performed on the effect of soluble chemokine and cytokine agents on the differentiation and phenotypic maintenance of cells, much less research has been focused towards elucidating the influence of physical stimuli on the underlying biochemical processes that guide differentiation and phenotypic expression.

Recently, several researchers have demonstrated the viability of using exogenous mechanical forces in the differentiation and guidance of cellular commitment towards several tissue types [1–6]. By means of engineered bioreactor systems, tensile, compressive, shear, and hydrostatic forces have all been demonstrated to activate various intracellular cascades that contribute to cellular differentiation [3,7,8]. However, we must consider that the application of exogenous force in the laboratory is not the only source of mechanical stress that the cells will experience during development. As a result of manipulation and, more importantly, as an inherent feature of adherent cell cultures, cells will be mechanically influenced by their attachment, migration and proliferation on the material chosen for their growth [9,10,11]. The microenvironment surrounding the cells is known to be one of the key factors that will ultimately determine their fate. Growth substrate chemistry will also condition the cells to express specific anchorage and junction molecules to ensure their adaptation to and growth on the material. For example, myofibroblast communication can be controlled through intercellular mechanical coupling via specific membrane bound structures [12]. Equally as important, exploiting growth material chemistry to guide the cells towards the *in vitro* expression of cardiac specific coupling molecules (such as connexin 43) can go a long way when it comes to their successful integration *in vivo* [13]. Finally, several studies have shown that the mechanical properties of the growth material itself can modulate and, in some cases, inhibit the differentiation of cells towards given lineages [14–17]. Researchers have demonstrated that cells in culture will modulate their own mechanical properties depending on the stiffness of the substrate they are grown on [18]. Similarly, the mechanical properties of the growth substrate have been shown to modulate the nature and composition of the integrin they assemble for their attachment [19,20].

If we consider the wide range of mechanical environments found in adult tissues within which

cells develop, maintain and in some cases regenerate the native structures, it is not unreasonable to hypothesize that control of mechanical properties of growth substrates *in vitro* can augment and/or modulate the resulting cellular response. Here we investigated the effect that growth on elastic silicone membranes has on the cardiomyogenic induction of periodontal ligament derived stem cells (PDLSC) by eliminating all other variables, including surface biochemistry.

2. Materials and Methods

2.1. Cell culture

Periodontal ligament derived stem cells were obtained as previously described [21]. Briefly, impacted 3rd molars were obtained from healthy donors following routine medical procedures requiring their extraction following our approved IRB protocols. The periodontal ligament was then dissected and enzymatically digested and filtered through a 40 μm cell strainer to obtain single cell suspensions. Cells were then selected for adherent dependence and cultured in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, Carlsbad CA) supplemented with 10% Fetal Bovine Serum (Invitrogen), 100 U/ml Penicillin streptomycin (Invitrogen) and 0.1% v/v amphotericin B (Fungizone; Hyclone, Logan UT). Passaging of the cells was achieved by enzymatic digestion in Trypsin/EDTA (Invitrogen) aided by mechanical disruption and subcultured as mentioned above. All cells used in the presented studies here were either passage 2 or 3.

2.2. Membrane preparation and seeding

Type 1 collagen coated silicone membranes were all purchased from Flexcell International (Flexcell International, Hillsborough NC) as large silicone sheets and cut into 0.7in × 1.75in strips using a printed template and sterile surgical scalpel. The membrane strips were then placed in Petri dishes and sterilized by overnight exposure to UV light inside a biosafety cabinet. PDLSC were seeded onto the membrane strips at a density of 10,000 cells/cm^2 in 750 μl total volume ensuring the cell suspension covered the entire span of the membrane. Cells were left to adhere overnight inside a tissue culture incubator and the media was changed the following day to remove any nonadherent cells. Cells were left undisturbed to proliferate on the membranes for 72 h prior to gene expression and immunohistochemical analyses.

2.3. Collagen 1 coating of tissue culture plasticware

In order to maintain equal surface biochemistry between the soft silicone membrane experimental group and the rigid plasticware control group, all plasticware used in these studies were coated with type 1 collagen solution (Sigma #C3867, St. Louis MO). Coating followed the manufacturer's recommended protocol. Namely the collagen solution was diluted to a working concentration of 0.01% using sterile, tissue culture grade water. Surfaces were coated with 10mg of total protein per centimeter squared (cm^2) of growth surface. Protein was allowed to bind overnight at room temperature and excess fluid was then removed and allowed to air dry. Finally, coated surfaces are sterilized by an overnight exposure to ultraviolet (UV) light inside a biosafety cabinet. Prior to cell culture, these surfaces were rinsed once with Phosphate Buffered Saline (PBS;

Invitrogen). Cells cultured on collagen coated plasticware were seeded at the same time and density as those seeded on silicone membranes.

2.4. RNA isolation, reverse transcription and polymerase chain reactions

2.4.1. RNA isolation

PDLSC total RNA was isolated by means of Trizol reagents following the manufacturers' recommended protocol. Briefly, cells were lysed and nucleic acids solubilized by means of homogenization in Trizol solution. Chloroform was then added to separate the RNA into an aqueous phase while leaving protein and DNA in the organic phase, containing chloroform. Aqueous phase was collected and RNA precipitated with 100% isopropanol and pelleted by centrifugation and the alcohol was then decanted. Finally, the RNA pellet was washed by addition of 75% ethanol, centrifuged again, allowed to air dry and resuspended in RNase and DNase free water. The RNA suspension was then frozen at –80 °C overnight and quantified using a NanoDrop® spectrofluorometer (Nanodrop Products, Wilmington DE).

2.4.2. Reverse transcription

Reverse transcription of RNA to cDNA was performed by means of the High Capacity cDNA Reverse Transcription Kit (cat #4374966; Applied Biosystems) as per the manufacturers' recommended protocol. Briefly, 1 μg of RNA for each sample was converted to cDNA by mixing it with the MuLV reverse trancriptase enzyme, a dinucleotide mixture (dNTP), random primers solution, RT reaction buffer and RNase inhibitors and set in a thermal cycler at 37 °C for 2 h followed by a quick inactivation at 85 °C for 5 minutes. Obtained cDNA solutions were then diluted to a final concentration of 10 ng/μl of PCR grade water and used for gene expression analysis.

2.4.3. Polymerase chain reaction (PCR)

Real time PCR was carried out using the TaqMan® Fast universal PCR Master Mix (Applied Biosystems) and TaqMan® probe pre designed primers for all the human genes explored (Table 1) along with 20 ng of cDNA for each sample. The reactions were performed using the Applied Biosystems StepOne Plus real time PCR system. Each reaction was run in triplicate (n = 3) for each assay and gene expression quantification was carried out by means of the comparative Ct method with normalization to GAPDH expression.

2.5. Immunohistochemistry analysis

Samples were first rinsed with PBS two times at room temperature and then fixed in a 10% formalin solution in PBS for 10 min at room temperature. Samples were then washed 3 times in PBS containing 0.05% v/v Tween 20 (Sigma). All samples were blocked using 1% BSA (containing 0.05% Tween 20) in PBS for 1 hour at room temperature. Samples were then incubated in primary antibody overnight at 4 °C. Following incubation with primary antibodies, the samples were washed twice in Tween 20 containing PBS and then incubated with the appropriate secondary antibodies (2 h

at room temperature). All antibodies were purchased from either Abcam (Abcam, Cambridge MA) or Santa Cruz (Santa Cruz Biotechnology, Santa Cruz CA). Following secondary antibody incubation, samples were again washed with PBS twice and counterstained for nucleic acids using DAPI (Invitrogen). Samples were then imaged using a Nikon Eclipse Ti inverted fluorescent microscope with the appropriate filters.

Table 1. List of genetic transcripts, Applied Biosystems catalog numbers, and reference sequences.

Transcript	Catalog #	Ref. Sequence
Glyceraldehyde-3-phosphate Dehydrogenase (GAPDH)	Hs99999905_m1	NM_002046.3
GATA Binding protein 4 (GATA4)	Hs00171403_m1	NM_002052.3
Myocyte Enhancer Factor 2C (MEF2C)	Hs00231149_m1	NM_001131005.2
NK2 transcription factor related, locus 5 (Nkx2.5)	Hs00231763_m1	NM_004387.3
Myosin light chain 2, cardiac, slow (MYL2)	Hs00166405_m1	NM_000432.3
Myosin, light chain 7, regulatory (MYL7)	Hs00221909_m1	NM_021223.2
Myosin heavy chain 7, cardiac, beta (MYH7)	Hs01110632_m1	NM_000257.2
Troponin T type 2 (cardiac) (TNNT2)	Hs00165960_m1	NM_001001430.1
Tropomyosin 1 (alpha) (TPM1)	Hs00165966_m1	NM_001018004.1
Connexin43/Gap junction protein α1 (Cx43)	Hs00748445_s1	NM_000165.3
Natriuretic peptide A (NPPA)	Hs00383231_g1	NM_006172.3
Natriuretic peptide B (NPPB)	Hs00173590_m1	NM_002521.2

2.6. Statistical analysis

All quantitative results are presented as mean ± standard deviation. Statistical analysis was performed by standard student t test for direct comparisons amongst experimental groups. Statistical significance was determined for analyses returning p values < 0.05.

3. Results

3.1. Growth on elastic silicone substrate upregulates gene expression of cardiomyogenic markers and contractile proteins

Analysis of the effect of silicone membrane growth on the cardiac specific genetic and protein expression by PDLSC revealed that the cells respond to growth on the softer elastic surface by upregulating some key myogenic markers following 4 days of growth on this substrate as compared to

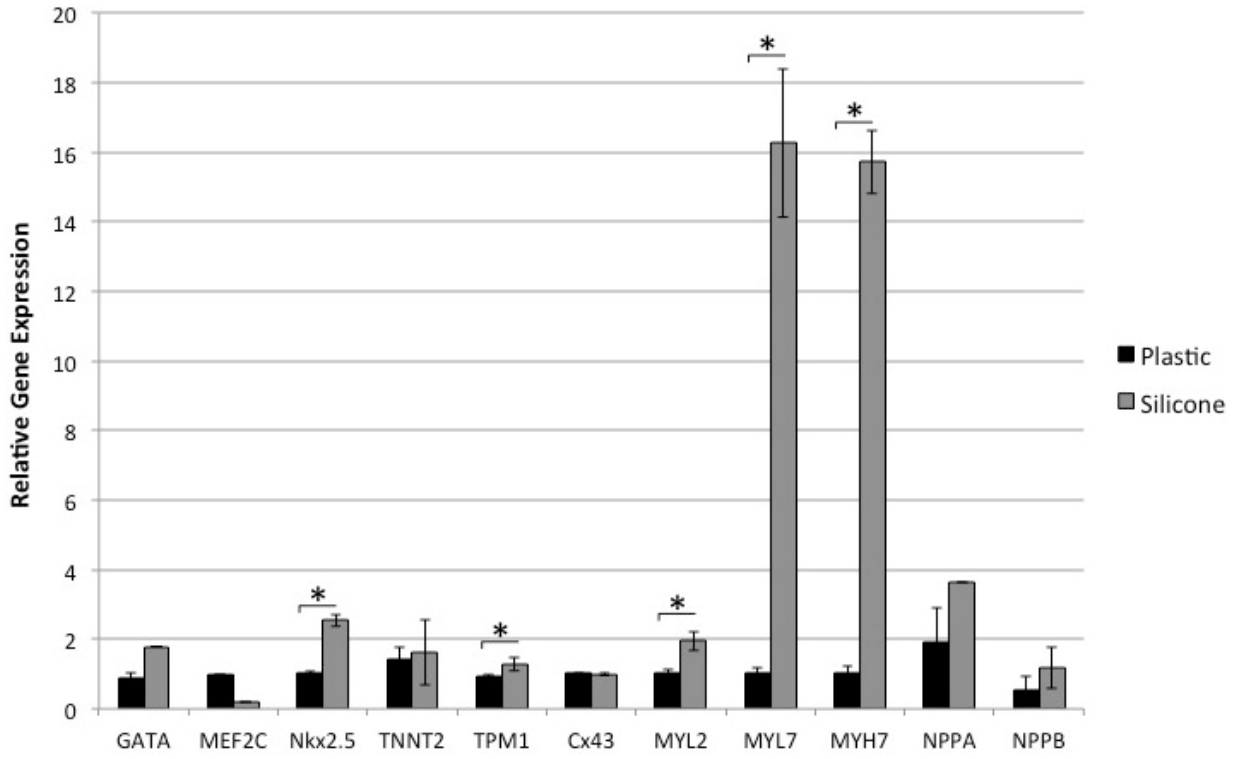

Figure 1. Cardiac specific gene expression analysis of Periodontal Ligament Derived Stem Cells (PDLSC) grown on either standard tissue culture plastic (Plastic) or the elastic silicone substrate (Silicone). Relative gene expression was determine by means of the comparative Ct method with normalization to GAPDH expression n = 3, *p < 0.05.

growth on collagen type 1 coated standard tissue culture plasticware. Genetic expression (Figure 1) reveals that PDLSC grown on an elastic silicone substrate significantly ($p < 0.05$) upregulate their expression of the Nkx2.5 transcription factor, a key regulator of cardiac myocyte differentiation. However, there was no statistical difference observed in the level of gene expression for the other two cardiac transcription factors analyzed, GATA4 and MEF2C. Similarly, gene expression for cardiac troponin T and connexin 43 were found to be not statistically different from that in cells grown on rigid plastic surfaces. On the other hand, the gene expression of the tropomyosin alpha 1 peptide (TPM1) was significantly upregulated in cells grown on the elastic substrate versus those on rigid plastic surfaces. This is the predominant tropomyosin isoform in striated muscle and is closely involved in the contractile potential of myogenic cells. Moreover, the greatest relative upregulation in gene expression for cells grown on the elastic substrate was observed in the genes coding for the functionally contractile myosin light chain polypeptides 2 and 7 (MYL2 and MYL7), and myosin heavy chain polypeptide 7 (MYH7). In fact, MYL7 and MYH7 experienced a greater than 10 fold increase in their level of expression in samples grown on the elastic substrate versus those grown on the plastic cultureware.

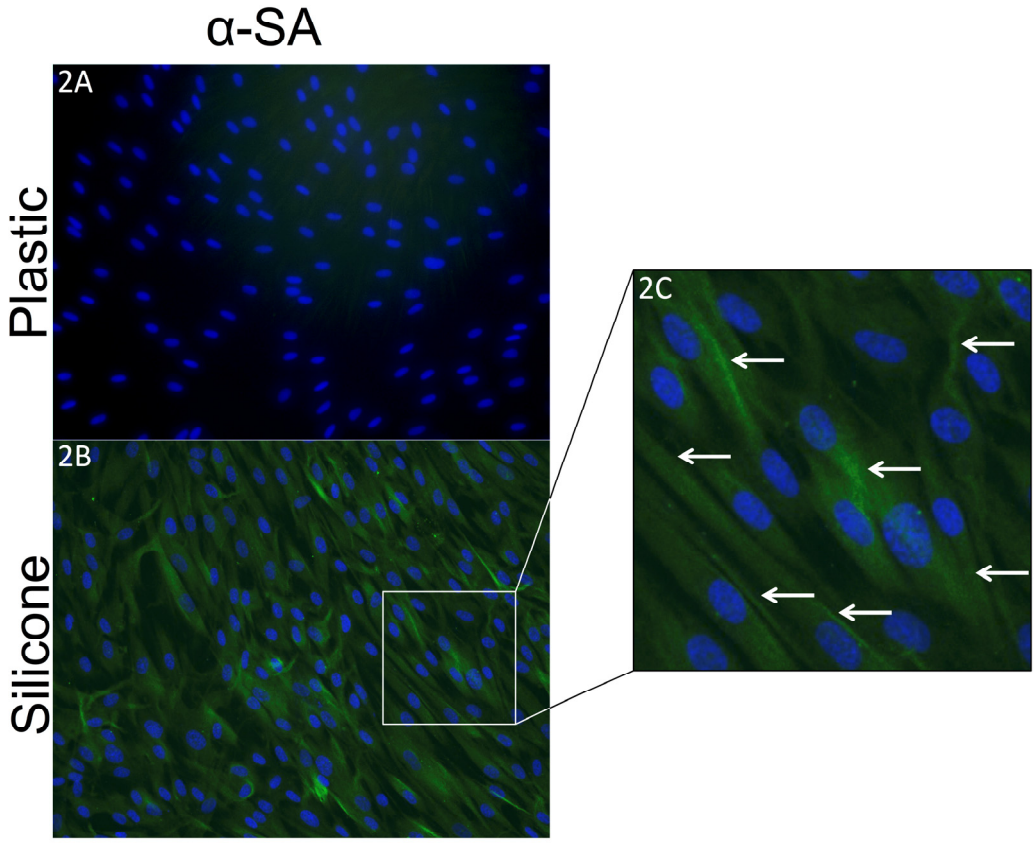

Figure 2. Immunohistochemical analysis of alpha sarcomeric actin (α-SA, green) counterstained with DAPI (blue) in Periodontal Ligament Derived Stem Cells (PDLSC) grown on either the standard tissue culture plastic (2A) or the elastic silicone substrate (2B). Figure 2C shows a region of the Silicone condition that is both magnified and highlighted for staining (white arrows).

3.2. Elastic substrate induces synthesis of myogenic proteins

At the protein level, immunohistochemical analysis shows similar results to those in the gene expression of cardiomyogenic markers (Figures 2 and 3). A modest increase in staining for the myogenic markers: alpha sarcomeric actin (Figure 2) and cardiac troponin T (Figure 3) is observed when cells are grown on the elastic silicone membranes versus rigid plasticware. However, it is to be noted that both the presentation and localization of these proteins is not morphologically correct for muscle tissues. Both alpha sarcomeric actin and cardiac troponin T positive expression is seen mainly in the perinuclear region, possibly at translational sites but not cytoplasmic wide, as would be expected. Similarly, the common striation of the actin fibers seen in mature myogenic tissues is not found in cells grown on the elastic substrate. Furthermore, despite the elevated genetic expression of the transcription factor Nkx2.5, no positive staining for the protein was detected during the immunohistochemical analysis of any of the samples (data not shown). These results suggest that, in conjunction with growth surface biochemistry, growth surface mechanical properties are an important variable in achieving or enhancing the proper differentiation of stem cells to desired phenotypes.

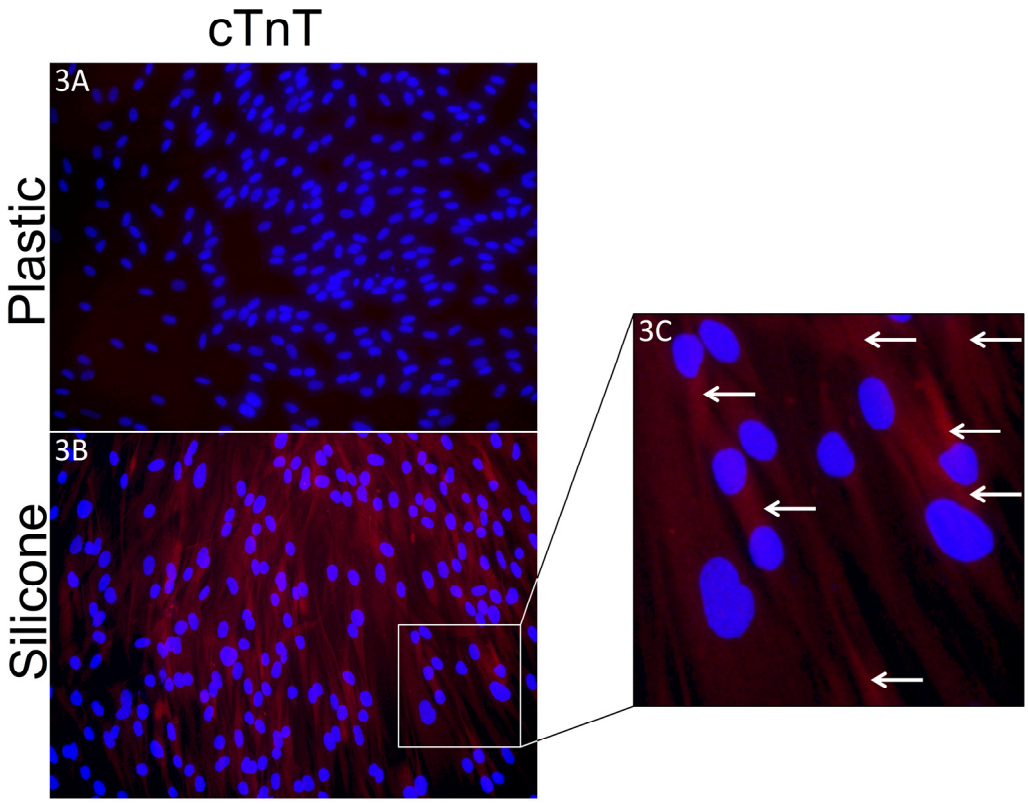

Figure 3. Immunohistochemical analysis of cardiac troponin T (cTnT, red) counterstained with DAPI (blue) in Periodontal Ligament Derived Stem Cells (PDLSC) grown on either the standard tissue culture plastic (3A) or the elastic silicone substrate (3B). Figure 3C shows a magnified region of the Silicone condition that points out cTnT staining (white arrows).

3.3. Morphological differences in cytoskeletal organization arise from growth on substrates with different mechanical properties

Fluorescent staining for the cytoskeletal filamentous actin (Figure 4) fibers shows a distinct morphological difference between cells grown on a rigid surface versus those grown on an elastic substrate. Cells grown on rigid plastic surfaces have the standard elongated spindle shape morphology of fibroblastic cells while cells grown on elastic silicone membranes have a flatter and shorter morphology to their cell bodies. The organization of the filamentous actin within these two groups of cells is also different. Most fibers seen within the cells grown on plastic plates have a clear overarching directionality spanning the cell body longitudinally through its longest axis. However, within any given cell grown on the elastic silicone substrate the arrangement of filamentous actin fibers is not completely uniform. While certain regions of directionality can be observed, fibers within the cells project in many directions spanning the cells length as well as width wise. On the other hand, Connexin 43 (the major GAP junction protein in cardiac muscle) expression remained high (a characteristic of the PDLSC used) and presented as diffuse cytoplasmic staining with areas of punctate staining in the distal cytoplasm and at cell/cell boundaries (Figure 5).

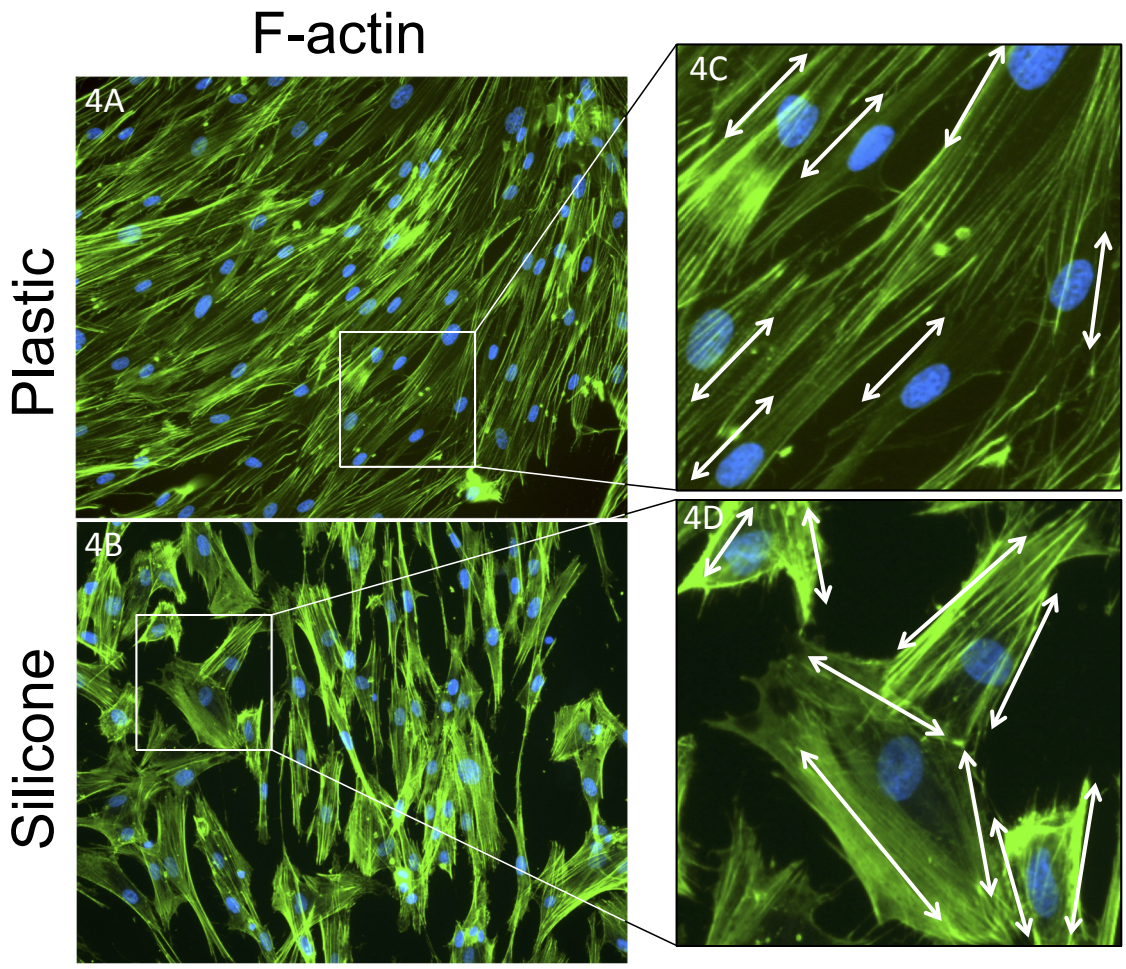

Figure 4. Immunohistochemical analysis of Filamentous actin (F-actin, green) counterstained with DAPI (blue) in Periodontal Ligament Derived Stem Cells (PDLSC) grown on either the standard tissue culture plastic (4A) or the elastic silicone substrate (4B). Figures 4C and 4D show magnified regions of both Figure 4A and 4B that highlight F-actin directionality (white arrows), respectively.

4. Conclusion

Our results show that growth on an elastic silicone substrate alone elicits a partial myogenic response from the cells. Statically grown cells on both plastic and silicone membranes in the present study were cultured on type 1 collagen coated surfaces, eliminating surface biochemistry as a variable for the observed results. These findings support the notion that a substrate's mechanical properties are essential parameters to consider when guiding cell phenotypic expression. When further analysis of the mechanical properties of the growth surfaces was performed, the silicone membrane was revealed to be at least 3×10^3 fold softer than the rigid tissue culture plastic. To further illustrate this point, one can compare the Young's Modulus values reported in the literature for murine cardiac muscle [22], skeletal muscle [23], and the two substrates employed here [9,24,25,26]. The tissue culture plastic is over 50,000 times stiffer than both muscle tissue specimens. While the elastic silicone membranes used in this study are 15 and 77 times stiffer than the values reported for myocardial [22] and skeletal muscle [23] respectively, the difference in

stiffness to a rigid substrate had a clear effect in overall cellular phenotype and morphology. Similar observations have been previously reported by several researchers, in which growth substrate mechanical properties have an effect on cellular phenotype [17,23,27,28]. In fact, Ren et al. [16], Engler et al. [15] and Gilbert et al. [23] all report the need for soft elastic substrates in the functionalization or enhancement of myogenic traits of different cells. While 2 out of 3 of these studies

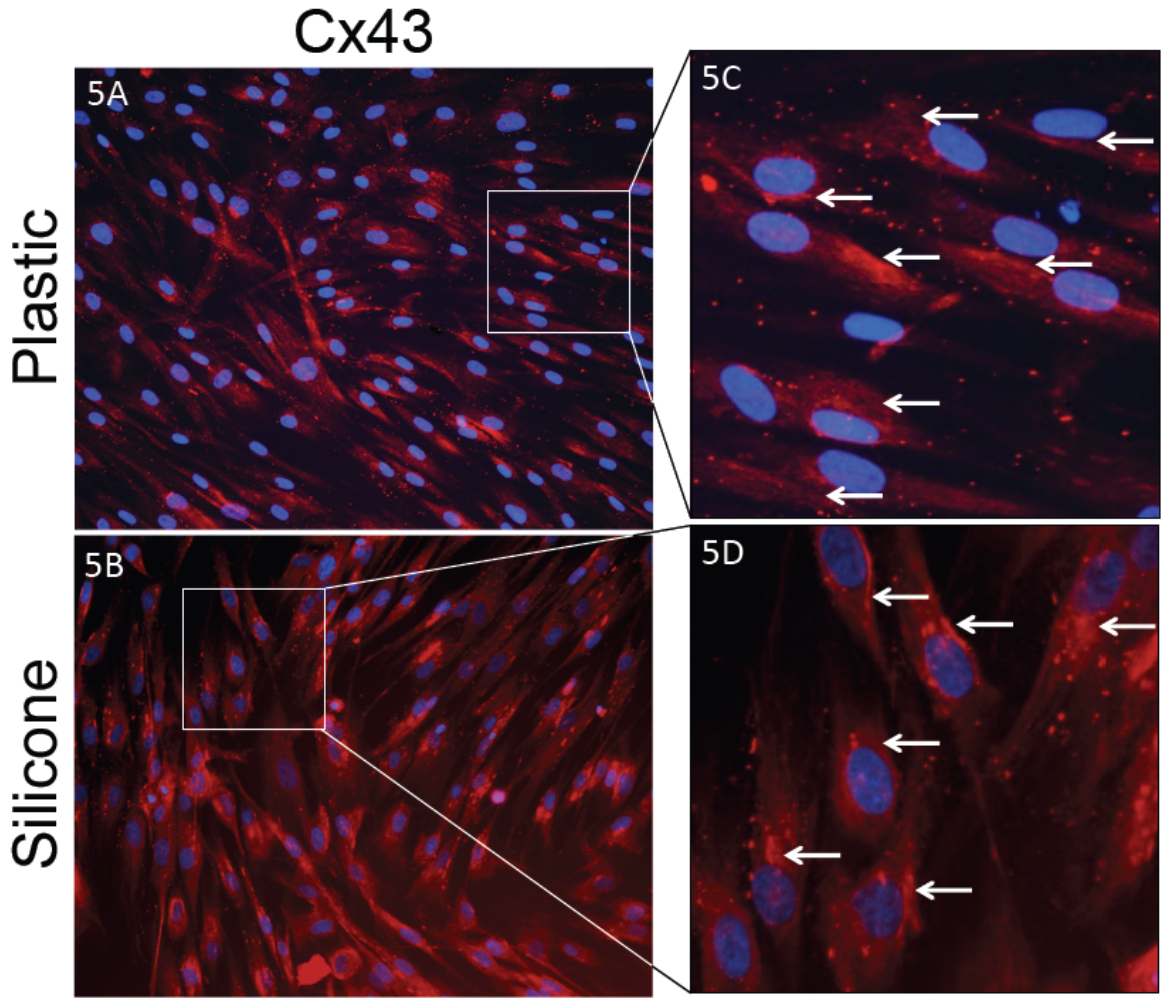

Figure 5. Immunohistochemical analysis of Connexin 43 (Cx43, red) counterstained with DAPI (blue) in Periodontal Ligament Derived Stem Cells (PDLSC) grown on either the standard tissue culture plastic (5A) or the elastic silicone substrate (5B). Figures 5C and 5D show magnified regions of both Figure 5A and 5B that highlight Cx43 staining (white arrows), respectively.

report that softer matrices are inductive of myogenic differentiation or self renewal, the study by Ren et al actually found that there is a limit to the softness of growth substrates as to the beneficial effects on myogenic phenotypic expression [16]. However, even the stiffer of the matrices employed by Ren et al, which induced correct cytoskeletal reorganization and increased proliferation of skeletal myoblasts were 10,000 times softer that the reported stiffness of plastic cultureware [9,24]. It is important to note, though, that these presented studies performed their experiments on different cell types and substrate biochemistries from one another, which might account for some of the reported

differences in their results. Furthermore, Solon et al [18] showed that fibroblasts were capable of adjusting their stiffness to match that of their surrounding environment with a saturation of rigidity occurring around 20 kPa. It was further shown that this modulation of stiffness was attributed to filamentous actin polymerization and degradation elicited by the mechanical properties of the growth scaffold rather than by its biochemical composition [18]. The aforementioned researchers have also investigated the mechanism that allows culture surfaces to influence cell phenotype. When surface biochemistry is held constant the primary mechanism of action involves the change of cell spread area and shape with material stiffness [23,27]. When cells are seeded on a material they will attach and pull on those attachments with a given force. As material stiffness decreases, the ability of cells to deform their environment increases, so the cells' surface areas decrease and their shape changes [28]. These effects were most clearly seen in Figure 4, where morphology of cells on silicone membranes became more compact and less fibroblastic. Therefore, we hypothesize that this morphological change directly influenced the myogenic response seen in both the gene expression and immunohistochemistry data.

Additionally, the concept of optimal mechanical properties of growth substrates may enhance the adaptability of cells preconditioned for the treatment of several clinical pathologies. For example, it has previously been shown that skeletal muscle stem cells retain more regenerative potential when cultured on less rigid materials. This knowledge may improve treatments for diseases such as muscle wasting syndrome by allowing one to culture more potent skeletal muscle stem cells for implantation [23]. In a similar manner, one may wish to more effectively culture cardiomyocytes for implantation in people with cardiomyopathies. Our research demonstrates that one may be able to achieve this goal by using a more elastic biomaterial. Further, we have shown that a modest cardiomyogenic response can be induced in stem cells without the presence of either soluble factors or mechanical strain. For researchers working in this field, it is particularly relevant knowledge that a non-committed cell type develops myogenic features due solely to a less stiff culturing surface. It is also very likely that the cardiomyogenic response our group observed might be further amplified by modifying silicone to more closely mimic muscle biochemical composition. At the very least, the presented study here argues for the need to consider the mechanical properties of the growth medium used in the maintenance, expansion and differentiation of cells that will eventually be employed in any regenerative medicine application.

Acknowledgements

This work was supported by the Dept. of Veteran Affair Merit Grant and Senior Research Career Scientist Award (HSC)

Conflict of Interest

All authors declare no conflicts of interest in this paper.

References

1. Shimizu N, Yamamoto K, Obi S, et al. (2008) Cyclic strain induces mouse embryonic stem cell differentiation into vascular smooth muscle cells by activating PDGF receptor beta. *J Appl*

Physio 104: 766–772.
2. Gwak SJ, Bhang SH, Kim IK, et al. (2008) The effect of cyclic strain on embryonic stem cell-derived cardiomyocytes. *Biomaterials* 29: 844–856.
3. Hwang YS, Cho J, Tay F, et al. (2009) The use of murine embryonic stem cells, alginate encapsulation, and rotary microgravity bioreactor in bone tissue engineering. *Biomaterials* 30: 499–507.
4. Pelaez D, Huang CYC, Cheung HS (2009) Cyclic compression maintains viability and induces chondrogenesis of human mesenchymal stem cells in fibrin gel scaffolds. *Stem Cells Dev* 18: 93–102.
5. Park JS, Huang NF, Kurpinski KT, et al. (2007) Mechanobiology of mesenchymal stem cells and their use in cardiovascular repair. *Front Biosci* 12: 5098–5116.
6. Kurpinski K, Chu J, Hashi C, et al. (2006) Anisotropic mechanosensing by mesenchymal stem cells. *Proc Natl Acad Sci USA* 103: 16095–16100.
7. Schmelter M, Ateghang B, Helmig S, et al. (2006) Embryonic stem cells utilize reactive oxygen species as transducers of mechanical strain-induced cardiovascular differentiation. *FASEB J* 20: 1182–1184.
8. Evans ND, Minelli C, Gentleman E, et al. (2009) Substrate stiffness affects early differentiation events in embryonic stem cells. *Eur Cell Mater* 18: 1–14.
9. Colley H, McArthur SL, Stolzing A, et al. (2012) Culture on fibrin matrices maintains the colony-forming capacity and osteoblastic differentiation of mesenchymal stem cells. *Biomed Mater* 7: 45015.
10. Li S, Huang NF, Hsu S (2005) Mechanotransduction in endothelial cell migration. *J Cell Biochem* 96: 1110–1126.
11. Rosenbluth MJ, Crow A, Shaevitz JW, et al. (2008) Slow stress propagation in adherent cells. *Biophys J* 95: 6052–6059.
12. Follonier L, Schaub S, Meister JJ, et al. (2008) Myofibroblast communication is controlled by intercellular mechanical coupling. *J Cell Sci* 121: 3305–3316.
13. Invernici G, Cristini S, Madeddu P, et al. (2008) Human adult skeletal muscle stem cells differentiate into cardiomyocyte phenotype in vitro. *Exp Cell Res* 314: 366–376.
14. Jacot JG, McCulloch AD, Omens JH (2008) Substrate stiffness affects the functional maturation of neonatal rat ventricular myocytes. *Biophys J* 95: 3479–3487.
15. Engler AJ, Carag-Krieger C, Johnson CP, et al. (2008) Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: scar-like rigidity inhibits beating. *J Cell Sci* 121: 3794–3802.
16. Ren K, Crouzier T, Roy C, et al. (2008) Polyelectrolyte multilayer films of controlled stiffness modulate myoblast cells differentiation. *Adv Funct Mater* 18: 1378–1389.
17. Winer JP, Janmey PA, McCormick ME, et al. (2009) Bone marrow-derived human mesenchymal stem cells become quiescent on soft substrates but remain responsive to chemical or mechanical stimuli. *Tissue Eng Part A* 15: 147–154.
18. Solon J, Levental I, Sengupta K, et al. (2007) Fibroblast adaptation and stiffness matching to soft elastic substrates. *Biophys J* 93: 4453–4461.
19. Paszek MJ, Boettiger D, Weaver VM, et al. (2009) Integrin Clustering Is Driven by Mechanical Resistance from the Glycocalyx and the Substrate *PLoS Comput Biol* 5: e1000604.
20. Pelham RJ, Wang YL (1998) Cell locomotion and focal adhesions are regulated by the mechanical properties of the substrate. *Biol Bull* 194: 348–350.

21. Huang CYC, Pelaez D, Dominguez-Bendala J, et al. (2009) Plasticity of stem cells derived from adult periodontal ligament. *Regen Med* 4: 809–821.
22. Hiesinger W, Brukman MJ, McCormick RC, et al. (2012) Myocardial tissue elastic properties determined by atomic force microscopy after stromal cell-derived factor 1α angiogenic therapy for acute myocardial infarction in a murine model. *J Thorac Cardiovasc Surg* 143: 962–966.
23. Gilbert PM, Havenstrite KL, Magnusson KEG, et al. (2010) Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science* 329: 1078–1081.
24. Avallone E, Baumeister III T, Sadegh A, (2007) N.A., In: N.A., *Marks' Standard Handbook for Mechanical Engineers*, 11 Eds., McGraw-Hill Education.
25. Material properties specification sheet: Bioflex membrane, 2006.
26. Katzengold R, Shoham N, Benayahu D, et al. (2015) Simulating single cell experiments in mechanical testing of adipocytes. *Biomech Model Mechanobiol* 14: 537–547.
27. Rowlands AS, George PA, Cooper-White JJ (2008) Directing osteogenic and myogenic differentiation of MSCs: interplay of stiffness and adhesive ligand presentation. *Am J Physiol Cell Physiol* 295: C1037–C1044.
28. Georges PC, Janmey PA (2005) Cell type-specific response to growth on soft materials. *J Appl Physiol* 98: 1547–1553.

AIMS Press