Mechanical properties of stem cells from different sources during vascular smooth muscle cell differentiation

Ruikai Chen, Delphine Dean
Department of Bioengineering, Clemson University, Clemson, South Carolina

Statement of Purpose: The purpose of our project is to investigate the mechanical property change during certain stem cell differentiation. Vascular smooth muscle cell (VSMC), as an essential component in human artery, plays an important role in regulating blood flow and pressure by contracting and relaxing in response to a variety of mechanical stimuli. Differentiation of stem cells into smooth muscle cell introduces a potential treatment for smooth muscle repair. A completely differentiated and functional vascular smooth muscle cell should have both the ability to contract and relax in response to environmental stimuli. The time period it takes for stem cells to fully differentiate into VSMC depends on many factors including the source of cells. In our study, we investigated stem cells from two different sources, bone marrow and adipose tissue. Changes in the differentiating cells' mechanical properties, namely elastic modulus, were determined by using atomic force microscope (AFM). Immunofluorescence and PCR were performed to determine if the expression of specific SMC markers are associated with mechanical property change. These results give us a brief view of elastic modulus change of VSMC differentiated from different stem cells and can be used to determine the optimal growth and environment required for VSMC in tissue repair and medical treatment.

Methods: Mesenchymal stromal cells (MSCs) isolated from human red bone marrow, were seeded at 1 X 10^4 cells/ cm^2 in 35-mm dishes and grown in MEM α, GlutaMAX (Invitrogen) supplemented with 20% FBS and 1% 100× penicillin-streptomycin. After 1 day of culture at 37C and 5% CO_2, differentiation media containing 10ng/ml TGF-β1 was added to induce differentiation to VSMC. Similar treatment was done to human adipose derived stem cells for differentiation. For AFM cytoindentation of cells, an Asylum Research MFP-3D AFM placed on a vibration isolation table was used. Borosilicate spherical probes with a nominal spring constant of 0.06N/m were utilized to indent into cells. The AFM probe was positioned centrally above the cell: the position was carefully adjusted to avoid the edge of the cell. The loading rate was set to 1μm/s to collect 5 indentation curves per cell. At each time point, 10 representative cells were chosen for evaluation and their elastic modulus were then analyzed using Hertzian linear elastic modeling. Immunofluorescence was performed on Paraformaldehyde (PFA) fixed cells with SMC-specific markers (α-actin, SM myosin heavy chain) and PCR analysis was conducted to quantitatively assess the expression of alpha-SMA during differentiation which is believed to be closely related to mechanical property change. In brief, RNA was extracted from different time point using Trizol reagent (life technologies). For alpha-SMA, the primers 5′-GCATCCACGAAACCACCTA-3′ and 5′-CACGAGTAACAAATCAAAGC-3′ were used while for β-actin as housekeeping gene, the primers 5′-TGGAATCCTGTGGCATCCATGAAAC-3′ and 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′were used to generate PCR product from cDNA.

Results: Overall, the process of vascular smooth muscle cell differentiation varies if given different sources of stem cells. Mesenchymal stem cells harvest from bone marrow tends to differentiate faster to smooth-muscle like cells compared to stem cells from adipose tissue. Smooth muscle-like cells differentiated from bone marrow stem cells also exhibits a much higher elastic modulus growth during differentiation. In general, the expression of specific SMC markers investigated by immunofluorescence matches up with AFM results. RT-PCR shows αSMA has a baseline expression for both undifferentiated mesenchymal stem cell and adipose stem cell along with a minor but constant increase over differentiation.

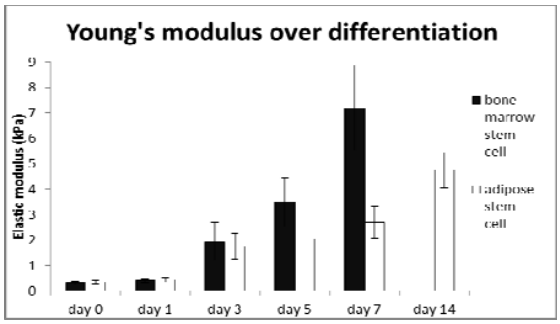
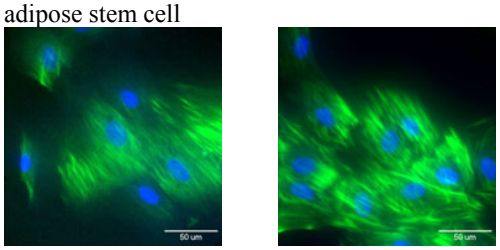


Fig. 1 Changes in Young's modulus during differentiation from day 1 to day 14 for bone marrow stem cell and adipose stem cell



(a) (b)

Fig. 2 Immunofluorescence imaging of calponin and nuclei for (a) day 7 adipose stem cell, (b) day 7 bone marrow stem cell. Scale bar =50μm

Conclusions: The result shows there is a significant difference between mechanical properties of differentiated vascular smooth muscle like cell from bone marrow and adipose derived stem cells while the expression of SMC specific markers match up with AFM results. Based on these data, we find the process of vascular smooth muscle cell differentiation varies if given different sources of stem cells and further investigations are required to better determine the appropriate mechanical environment for vascular smooth muscle differentiation used in tissue engineering application.

References: 1. Wang, C., S. Yin, et al. *Tissue Engineering* Part A 16(4): 1201-1213.