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In this study, we have shown the importance of cell geometry in cellular response to mechanical stress and have validated this using three different approaches. By manipulating geometry, we have been able to show that cell geometry not only affects gene expression but also affects the orientation of nuclear stress fibers, and this in turn modulates mechanical forces on the chromatin. Our study also demonstrates that cells with different geometries respond differently to mechanical force and this results in different gene expression changes. This work can be extended to demonstrate the importance of cell geometry in controlling chromatin organization and gene expression in vivo in response to mechanical forces. The results indicate that the regulation of nuclear-cytoplasmic asymmetry by the application of compressive force on the ECM is determined by the geometry of the fibroblast and could be an important determinant in cellular migration and invasion. Our study is the first demonstration that small intrinsic changes in cellular geometry can have a profound impact on the transcriptional response. In addition, these observations suggest that the use of cells of different geometries on orthotropic materials could lead to different gene expression patterns that could be potentially useful for cell-based applications such as tissue engineering. The implications of the results are to demonstrate that cell geometry is important not just in pathological conditions like cancer but also in engineering cell-based therapies. Our work highlights the importance of cell geometry, along with mechanical forces in determining cellular and nuclear structure and how it regulates transcription. While external compressive force alters the internal pressure that the nucleus experiences, the internal pressure is dependent on both the geometry and the mechanical properties of the nucleus.

## Geometry Expressions 3.0.8 ML.rar

In this study, we test the expression levels of three commonly used reference genes (GAPDH, HMBS and RPL13A) in mature primary ASCs of different donors and in their associated preadipocytes ( Supplemental Sections SD-SI, SII and SIII ). We observe similar expression levels for all three genes in preadipocytes, monolayers, monolayers on compressive forces, and circular monolayers of different shapes. On the other hand, when compared with monolayer, the monolayer on a compressive forces and circular monolayers exhibit higher expression levels for all three genes ( Figure S2 A). These observations suggest that normalizing to these three commonly used housekeeping genes could yield erroneous results in ASCs. As the expression level of a gene of interest is often inversely correlated to that of housekeeping genes, we tested the expression levels of three commonly used adipogenesis-associated genes (FABP4, PPAR $\gamma$  and LEP) in mature ASCs of different donors and their associated preadipocytes. We observe reduced expression levels for all the three genes ( Figure 2 C). We next investigated the expression levels of these three genes in ASCs during their differentiation. We observe an increase in their expression as indicated by normalized C(T) values ( Figure 2 D). Quantitative PCR is often chosen as a method for determining gene expression in gene expression studies. However, as with every method, the results obtained from qPCR is dependant on the choice of reference gene. However, this is often overlooked and could often lead to erroneous results. Reference genes are often chosen based on their stability over time. This is always a concern for real time PCR. Having a housekeeping gene that is up-regulated during differentiation helps in reducing the risk of error in gene expression determinations. We tested expression levels of three commonly used reference genes during ASC differentiation, that is, GAPDH, HMBS and RPL13A ( Supplemental Sections SD-SI, SII and SIII ). We find all three genes to be up-regulated during monolayer-based and compressive force-mediated differentiation, but to a lesser extent compared to the monolayer-based differentiation. On the contrary, gene expression levels remain unchanged for all the three genes during differentiation on fibronectin-coated circles ( Figure S2 B). We find that changing the shape of the cells alters the expression level of these commonly used genes. For example, GAPDH and HMBS expressions are significantly reduced in cells on micropatterned circles compared to the monolayers. On the other hand, RPL13A expression remains unchanged ( Figure 2 E). Furthermore, we also find HMBS expression levels to be reduced in the presence of either Wnt3a or the inhibitor XAV939 ( Figure 2 F). These observations demonstrate the importance of selecting appropriate reference genes, and point towards GAPDH and HMBS as the optimal choice for ASC studies. 5ec8ef588b

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