Biophysical microenvironmental factors have shown to couple and interact to regulate various cell behaviors [1,2]. Although plenty of phenomenological work has been done on different cell types to investigate microenvironmental sensing. A deep and comprehensive understanding of how biophysical conditions affect the cellular transcriptome is lacking.

In this study, we harness RNA sequencing as a computational tool to thoroughly screen the response of the cellular transcriptome of mouse mesenchymal stem cells (mMSCs) to different combinations of biophysical cues, including stiffness, stress relaxation and adhesion ligand density. A 3D alginate culture system was formulated from the results of the functional enrichment analysis. We identified and functionally analyzed the most significant DE genes of mMSCs under different combinations of biophysical cues. A list of DE genes was extracted and visualized for every pairwise comparison. Overall, the results agree with the conclusions of the original paper [3]. We attribute the discrepancy of results to the following factors. 1) Different analysis tool and settings, 2) Sample variations, 3) Different gene annotation files used.

**Background**

- **Methods & Design**
  - The raw data of the study comes from a published paper from Dr. David Mooney’s lab at Harvard University [3].
  - A 3D alginate culture system was engineered to independently control biophysical parameters. mMSCs were encapsulated in eight conditions: low or high values of stiffness, stress relaxation, and ligand density.
  - A pairwise comparison of each condition was performed to identify the most significantly differentially expressed genes.
  - Using computational packages from the Galaxy platform [4], we aligned the sequences to the annotated genome (HISAT2), quantified gene counts (StringTie), and analyzed significant changes in gene expression between groups (DESeq2).
  - Functional enrichment analysis (g:Profiler) maps genes to their related biological processes and functional pathways [5].

For each pairwise comparison, four plots were used to visualize the distribution of gene counts. Clear separation of clusters in principle component analysis, large sample-to-sample distance, high frequencies of p-values lower than 0.001, and high log fold changes compared to mean normalized counts (genes labeled in red) indicate larger numbers of and higher extent of differentially expressed genes between the two conditions. The below two comparisons show drastically different behaviors.

- **Results**
  - Fast vs. Slow relaxation, 38kPa, High Ligand Density
  - Fast vs. Slow relaxation, 38kPa, Low Ligand Density

The below heatmap shows a large discrepancy in the number of differentially expressed genes and their up/down regulation under different parameter conditions. Stiffness > Stress Relaxation > Adhesion Ligand Density in influencing #DE genes at the conditions of this study.

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**Future Directions**

- Further functional enrichment analysis could be done using Metacore to construct putative gene networks and identify models of gene analogously affected by biophysical conditions.
- Various hypothesis linking cell function to biophysical cues may be formulated from the results of the functional enrichment analysis. However, one must be mindful of the cell-type and biophysical parameter range dependence of cellular behaviors.
- PCSK6 is upregulated in human ath erosclerotic plaques associated with extracellular matrix remodeling [7]. Mechanistic studies could be done to investigate how stress relaxation regulates intimal hyperplasia progression.
- IBSP is a cancer gene that promotes tumor metastasis through epithelial mesenchymal transition (EMT) [8]. 3D hydrogel models could be constructed to study EMT and screen for cancer cell relaxation conditions could be constructed to study EMT and screen for cancer therapeutics.

**References**