Systems Biology/Bioinformatics and Characterization of Stem Cell-Based Cell Therapy Products

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Primary Human Bone Marrow Stromal Cells (hBMSCs)

- Tex A&M Center, Darwin Prockop (Tulane/NIH)
- Iliac crest harvest
- 29 yr old female

Positive (> 95%)
- CD105
- CD73
- CD90

Negative (< 2%)
- CD45
- CD34
- CD14
- CD11b
- CD79a
- CD19
- HLA-DR

Osteogenic Suppl.:
- Dexamethasone
- Ascorbic Acid
- β-Glycerophosphate

Primary Human Bone Marrow Stromal Cell (hBMSC) Osteogenic Differentiation: Alizarin Red Stain for Calcium

Result: Nanofibers induce calcification
Microarray Experiment (mRNA)

Experimental Design

- 72 Specimens = 72 Microarrays
- hBMSCs for all exps
  - 4 Replicates
  - 2 Times Points (1d, 14d)
  - 9 Substrates
    - TCPS
    - TCPS+OS
    - PCL_FFF
    - PCL_GF
    - PCL_BNF
    - PCL_SC
    - PCL_SNF
    - PDLLA_BNF
    - PDLLA_SC

Illumina Human HT-12v4 Microarrays

- 47231 probes
- 25130 RefSeq annotated genes (NCBI/NIH)
Microarray Experiment (mRNA)

- Sort by treatment
- Nanofibers similar to osteogenic controls
- Structure more important than chemistry (?)
Why Do Nanofibers Induce Osteogenic Differentiation? Cell Shape…

- Nanofibers & Films+OS = elongated & highly branched
- Films = hBMSCs more spread, more rounded & less branched
- Can drive shape change with scaffold structure or biochemicals (?)
hBMSCs in Nanofiber Scaffolds

1 d, PDLLA_BNF spiked with Rhodamine 123

Red = Actin
Green = Nanofibers

Effect of Nanofiber Scaffolds on the hBMSC Proteome

People: Tanya Farooque, Subhadip Bodhak, Sumona Sarkar, Michail Alterman, Kristin Schultz-Kuszak

Aims:
• Map the proteomic signature of hBMSCs
• Protein expression patterns during cell culture in 3D
• Compare predictive ability of transcriptome vs. proteome

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Microarray Experiment Not Reproducible

**1st Experiment**
Donor 7038, 29 y female
853 genes passed the 20% 1.5-fold filter

**2nd Experiment**
Donor 8001R, 24 y female
89 genes passed the 20% 1.5-fold filter

**Conclusions:**
- hBMSCs sort by treatment in both cases
- Nanofibers don’t sort with TCPS(+)OS in both cases
- Donor 8001 less responsive than Donor 7038

**3rd Experiment:** 6 donors, 4 treatments, 1 replicate
hBMSC Morphology & hBMSC State

43 Shape Descriptors

- Angle
- Area
- Polygonal Area
- Area/Box
- Aspect
- Axis (major)
- Axis (minor)
- Box Height
- Box Width
- Box Ratio
- Dendrites
- Dendritic Length
- Maximum Diameter
- Mean Diameter
- Minimum Diameter
- End Points
- Maximum Feret Length
- Mean Feret Length
- Minimum Feret Length
- Fractal Dimension
- Cell Area/Total Area
- Perimeter
- Perimeter2
- Perimeter3
- Convex Perimeter
- Elliptical Perimeter
- Perimeter Ratio
- Maximum Radius
- Minimum Radius
- Radius Ratio
- Roundness
- Size (Length)
- Size (Width)
- Mean Density
- Standard Deviation of Density
- Sum of the Density
- Integrated Optical Density
- Holes
- Hole Area
- Hole Ratio
- Margination
- Heterogeneity
- Clumpiness

High-content imaging, 43 shape descriptors, condense non-linearly into 3 dimensions and segment

Cells in osteogenic medium segment from adipogenic or basal medium

Cell Volume: Small hBMSCs More Potent

PCBM1641 at P7 sorted by volume:
- Large = 19.3 μm = 1/296 (+) for adipogenesis
- Small = 14.6 μm = 1/126 (+) for adipogenesis
- p = 0.02

Donor JL, Bauer SR. Tiss Eng C, v18, p877, 2012

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<tr>
<th>Donor #</th>
<th>ID #</th>
<th>Sex</th>
<th>Age (Years)</th>
<th>Diameter (Mean ± S.D.) (µm)</th>
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<td>7038</td>
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<td>29</td>
<td>19.0 ± 0.3</td>
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<td>8001</td>
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<td>18.6 ± 0.2</td>
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<td>Donor 5</td>
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<td>Female</td>
<td>37</td>
<td>17.7 ± 0.0</td>
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Conclusions & Future Directions

Conclusions
• Nanofibers enhance osteogenic differentiation
• Scaffold structure can be optimized to drive hBMSCs into morphologies that enhance differentiation
• Donor variability very important variable

Future:
• Proteomics
• Multi-donor microarray experiment
• Cell shape and machine learning
• Cell volume
• Integrome
• Connectome = Integrome + Cell Shape Metrics
Cell Shape & Advanced Computation
Scaffold Structure Directs Stem Cell Fate

Images are from Kumar et al., 2011
Geometry-Driven Stem Cell Differentiation

- We would like to understand how geometry of the substrate induces stem cell differentiation
- Could design substrates to achieve desired differentiation
- Large number of parameters need to be tested for identifying appropriate scaffolds
- Could also be used in scaffold systems for drug screening by pharmaceutical industry
  - Cell Shape analysis could be used to determine toxicity response
Computational Regenerative Medicine

Need to develop a framework for Big-Data-Driven Regenerative Medicine

- High-throughput Imaging
- High-performance Computing and Visualization
- Geometry-driven Quantitative Analysis
Data-driven Classification of Stem Cells

Data Source:

- We used stacks of confocal microscopy images of size 2048 x 2048 x ~20
- Our sample set contained 41 cells, but future drug discovery applications may have 1000s of cells

Classification of Stem Cells

- Cells with more thin branches are expected to have a larger number of short intersections
- Need an algorithm that is easily parallelizable for high throughput data computing
Computing on CPUs and GPUs

- High memory bandwidth
- High number of cores
- High computational capability
- Partitioning the computational task between CPUs and GPUs
In Silico Cell Shape Analysis

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Comparison of algorithm running times. The CPU algorithm was run on an Intel Xeon X5260 (using only one core) with 8 GB of RAM. The GPU algorithms were run on an NVIDIA Tesla C2050 (448 cores)
Data-driven Learning

Once histograms are generated, they can be used to train an SVM classifier, which can then be used to classify new cell histograms as Nanofiber or Spun Coat.
Classification Results

Classification accuracy with our test data set was over 80%

![Bar chart showing percent correct versus number of lines. The x-axis represents the number of lines, ranging from $10^1$ to $10^6$, and the y-axis represents the percent correct, ranging from 0 to 100. The bars indicate an increasing trend as the number of lines increases.](image-url)
Conclusions and Future Work

- Initial steps towards a computational imaging pipeline for stem cell differentiation analysis

- Need further research on better characterization of relationships between scaffold geometry and stem cell morphology

- Big data driven computing can play a significant role in development of quantitative techniques to assist in regenerative medicine
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<td>Girish Kumar (FDA)</td>
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<td>Tanya Farooque (FDA)</td>
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<td>Michail Alterman (FDA)</td>
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<td>Kristin Schulz-Kusak (FDA)</td>
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