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

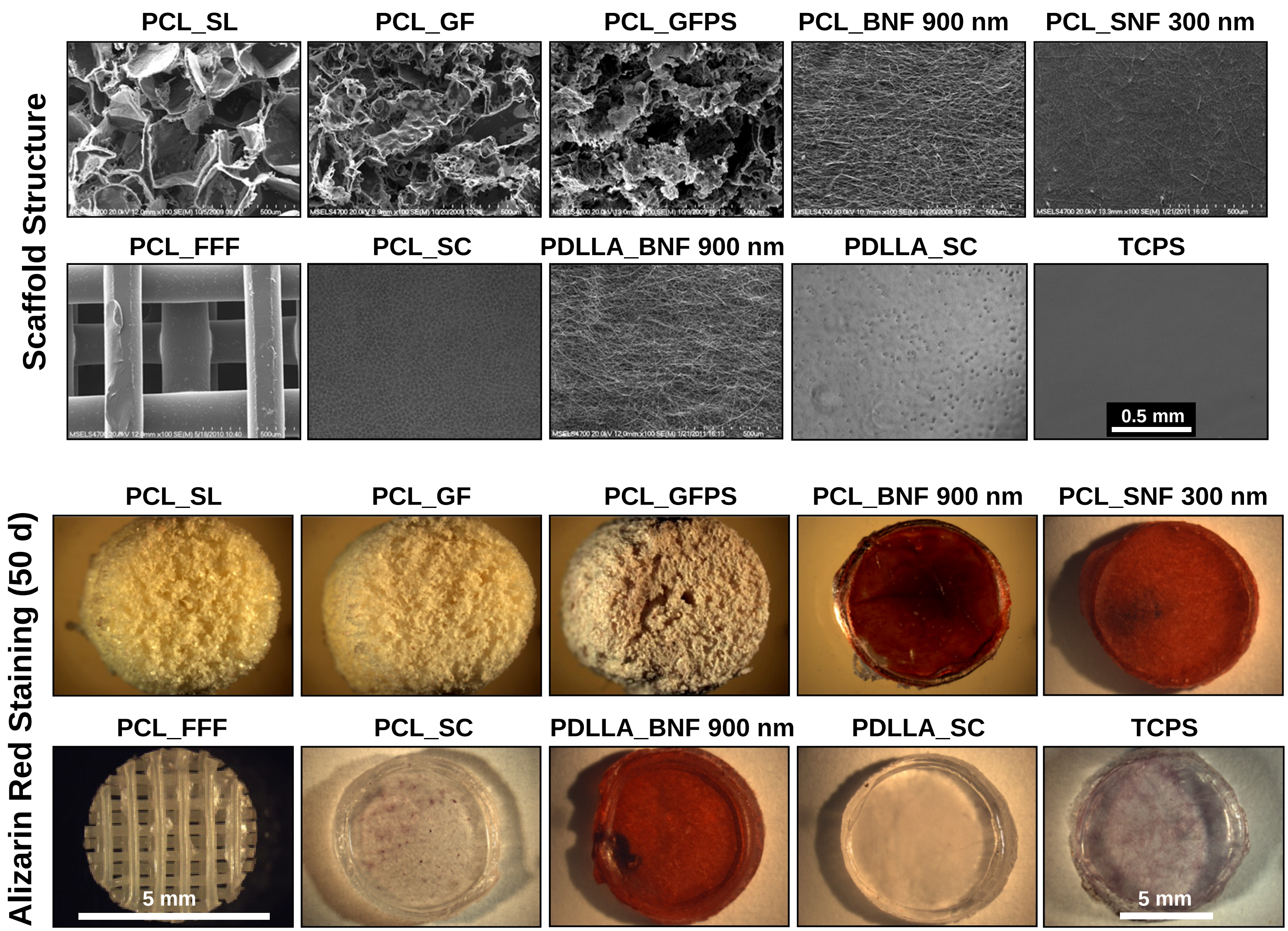
Positive (> 95%)
- CD105
- CD73
- CD90

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

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

Dominici et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy, 8:4, 315-317.

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



Result: Nanofibers induce calcification

Microarray Experiment (mRNA)

Experimental Design

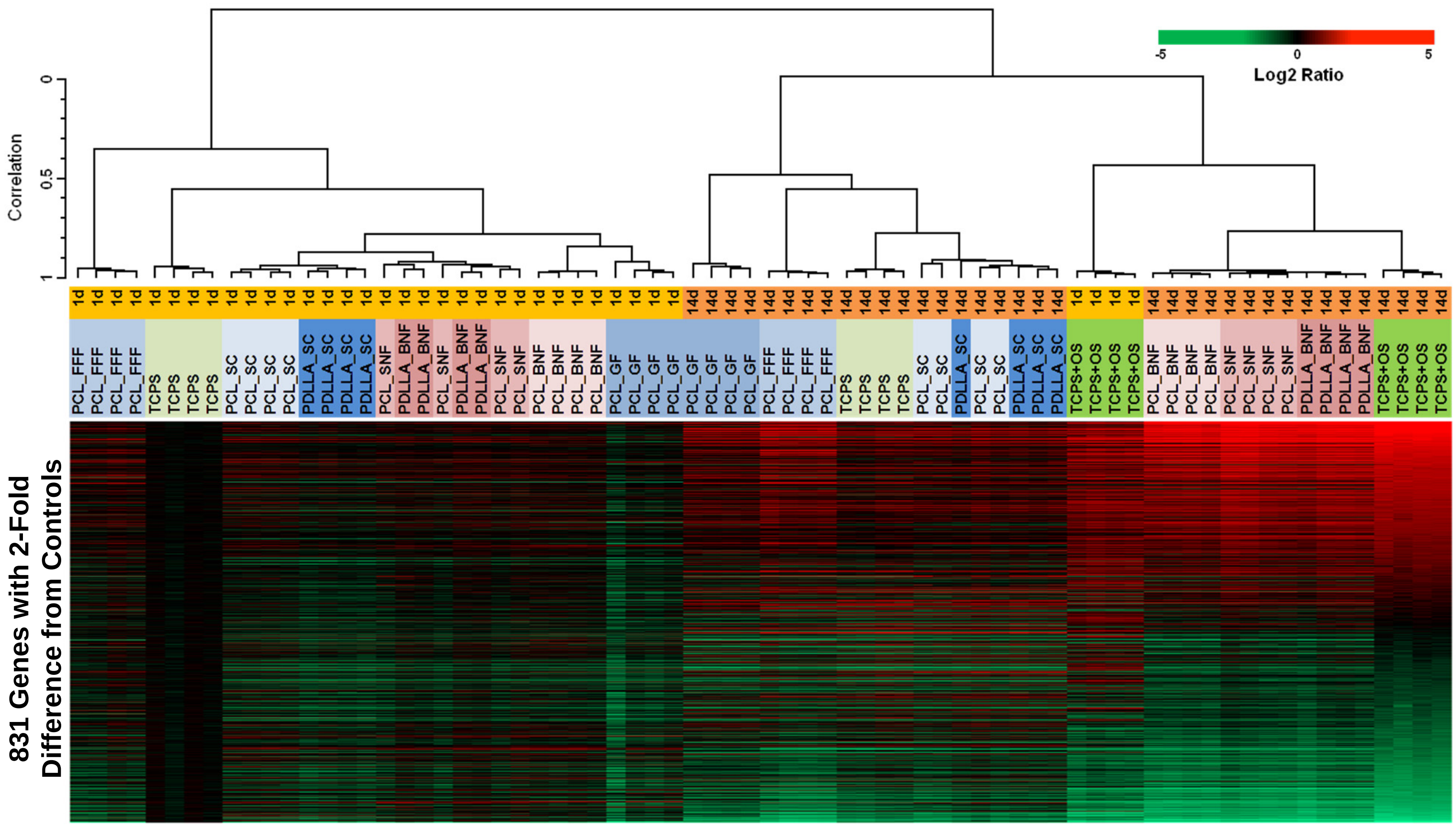
- 72 Specimens = 72 Microarrays
- hBMSCs for all exps
  - 4 Replicates
  - 2 Times Points (1d, 14d)
  - 9 Substrates
    - TCPS
    - TCPS+OS
    - PCL_FFF
    - PCL_GF
    - PCL_BNF
    - PCL_SC
    - PCL_SNF
    - PDLLA_BNF
    - PDLLA_SC

illumina

Illumina Human HT-12v4 Microarrays

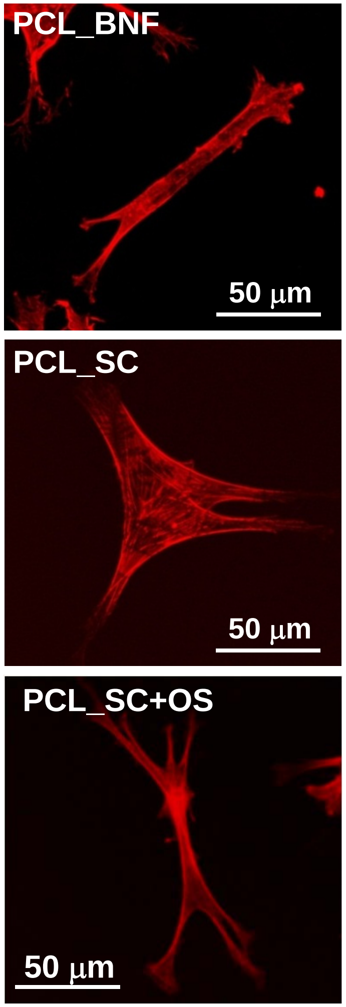
- 47231 probes
- 25130 RefSeq annotated genes (NCBI/NIH)

Microarray Experiment (mRNA)

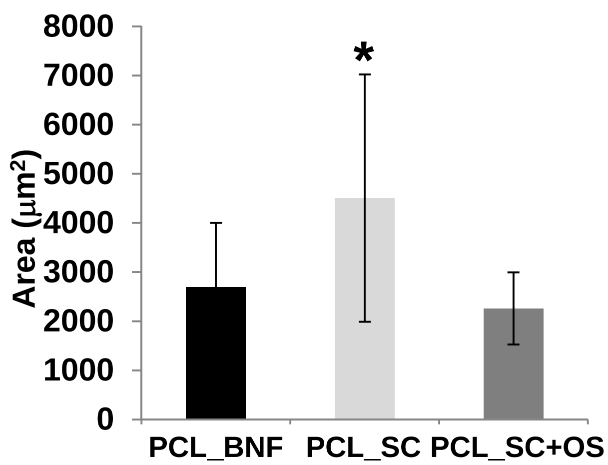


- **Sort by treatment**
- **Nanofibers similar to osteogenic controls**
- **Structure more important than chemistry (?)**

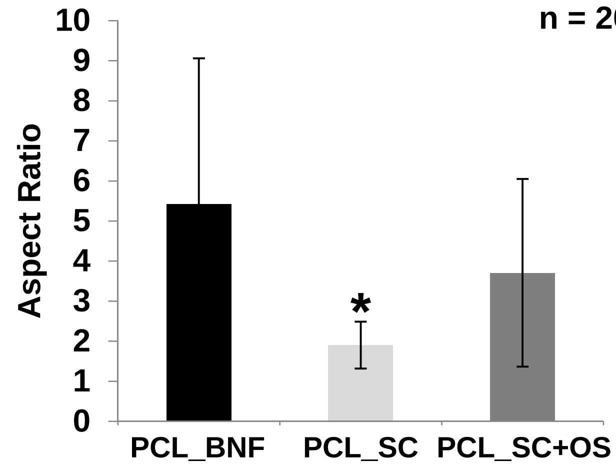
Why Do Nanofibers Induce Osteogenic Differentiation? Cell Shape…

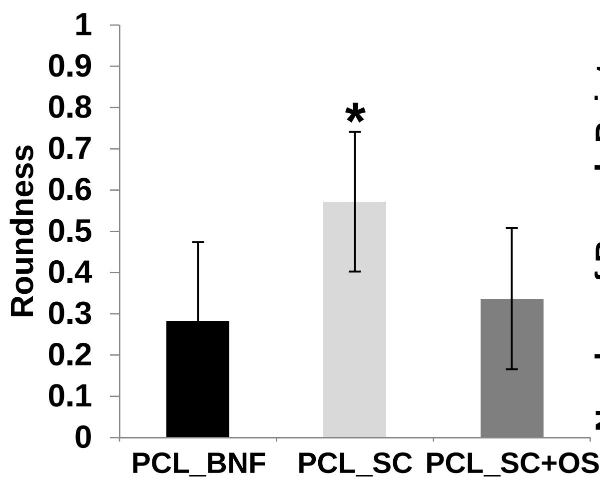


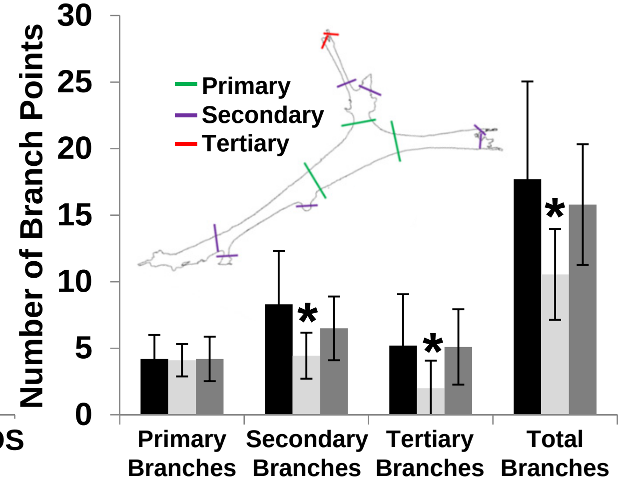
Red = Actin



n = 20 Cells

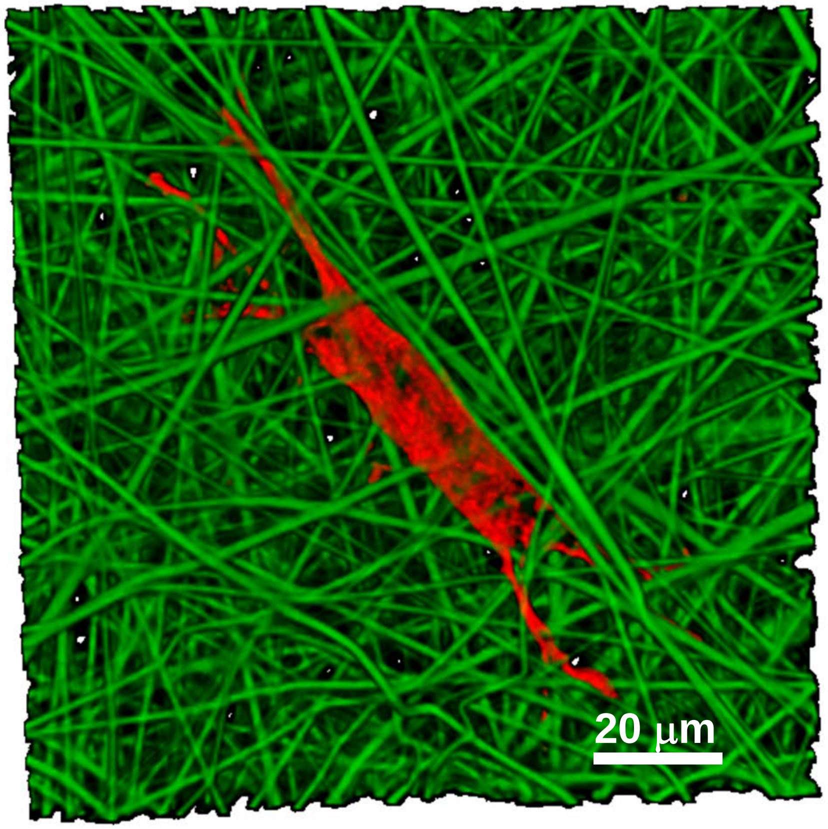




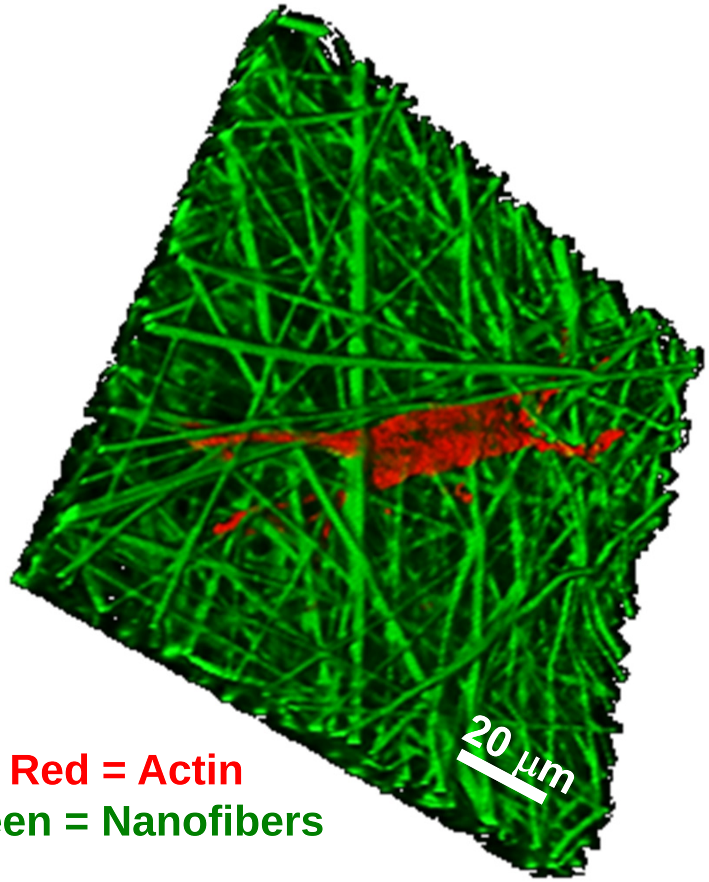


- Nanofibers & Films+OS = elongated & highly branched
- Films = hBMSCs more spread, more rounded & less branched
- Can drive shape change with scaffold structure or biochemicals (?)

hBMSCs in Nanofiber Scaffolds



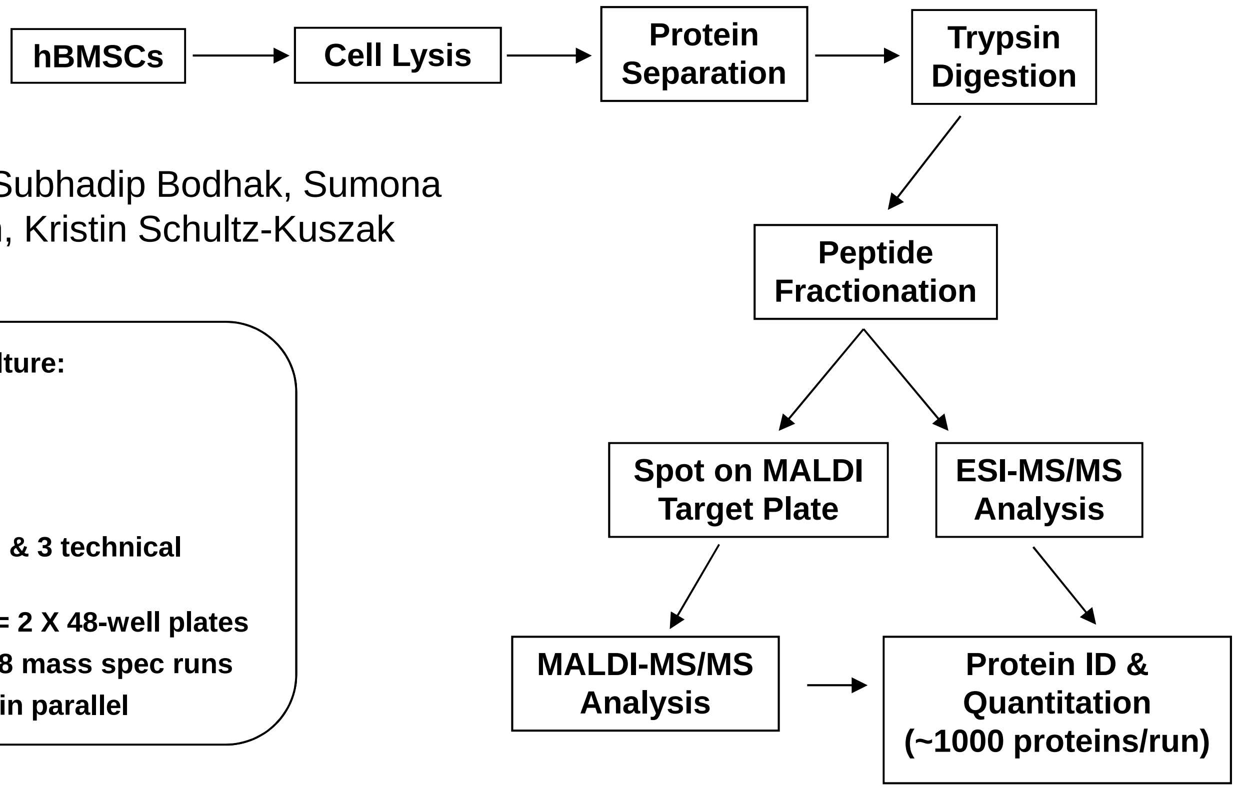
1 d, PDLLA_BNF spiked
with Rhodamine 123



Red = Actin
Green = Nanofibers

Kumar G, Tison CK, Chatterjee K, Pine PS, McDaniel JH, Salit ML, Young MF, Simon Jr CG (2011) The determination of stem cell fate by 3D scaffold structures through the control of cell shape. Biomaterials 32, 9188-9196.

Effect of Nanofiber Scaffolds on the hBMSC Proteome



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

- **4 Treatments, 14 d culture:**
  - **PCL-NF**
  - **PCL-SC**
  - **TCPS**
  - **TCSP (+)OS**
- **2 biological replicates & 3 technical replicates**
- **1 biological replicate = 2 X 48-well plates**
- **Total = 1600 wells & 48 mass spec runs**
- **Running microarrays in parallel**

Aims:

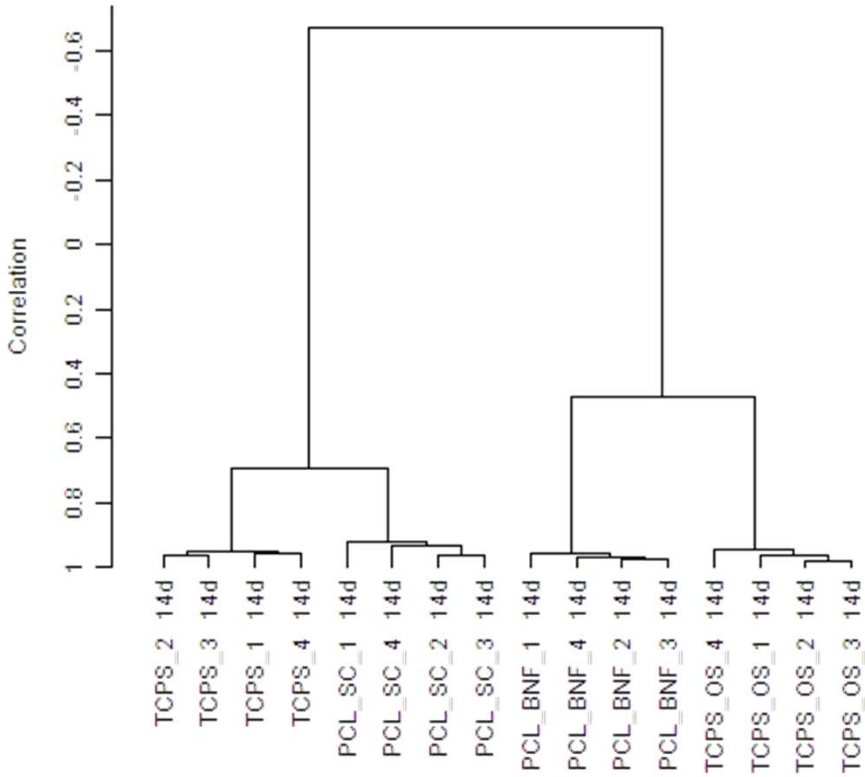
- Map the proteomic signature of hBMSCs
- Protein expression patterns during cell culture in 3D
- Compare predictive ability of transcriptome vs. proteome

Microarray Experiment Not Reproducible

1st Experiment

Donor 7038 , 29 y female

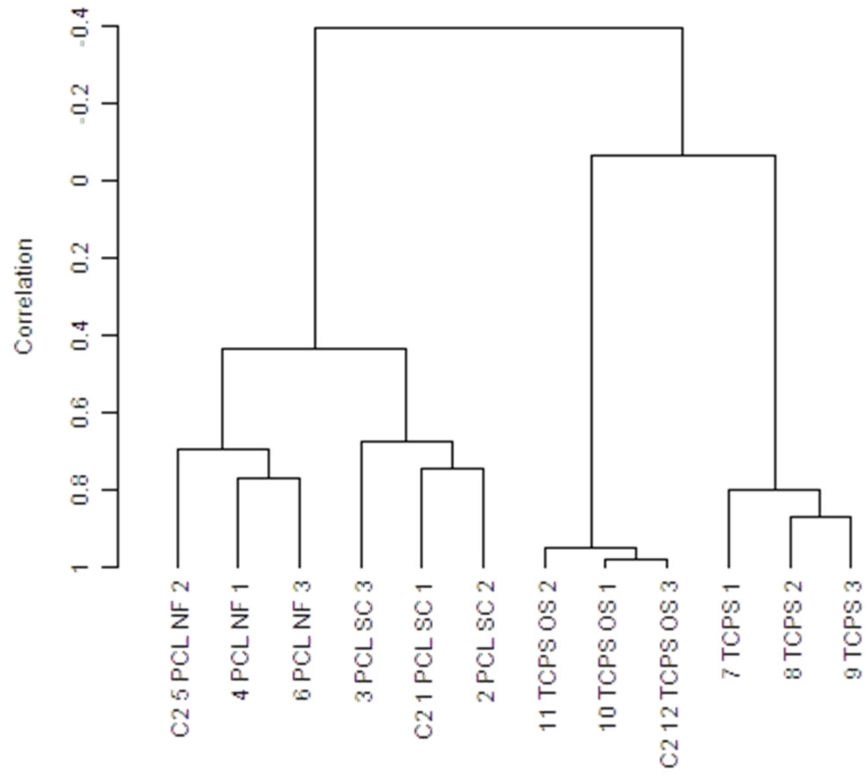
853 genes passed the 20% 1.5-fold filter



2nd Experiment

Donor 8001R, 24 y female

89 genes passed the 20% 1.5-fold filter

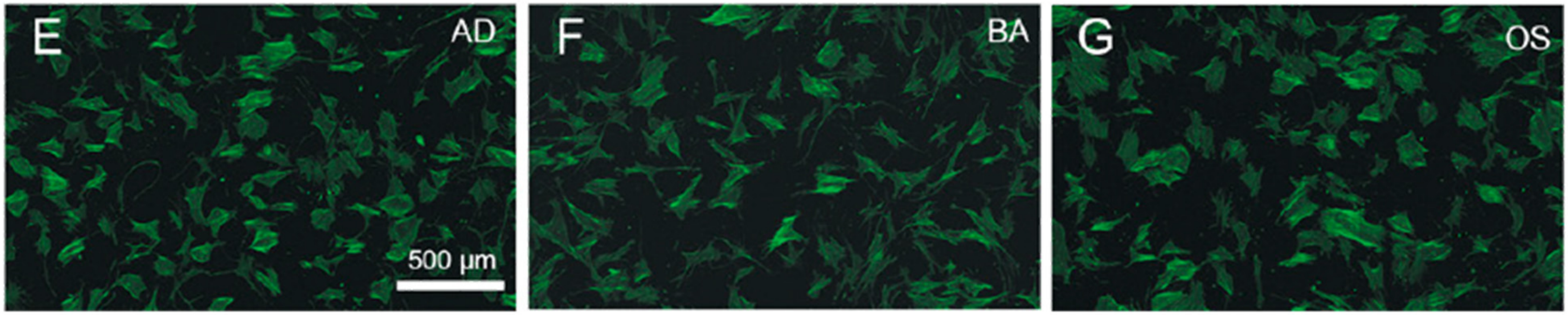


Conclusions:

- 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

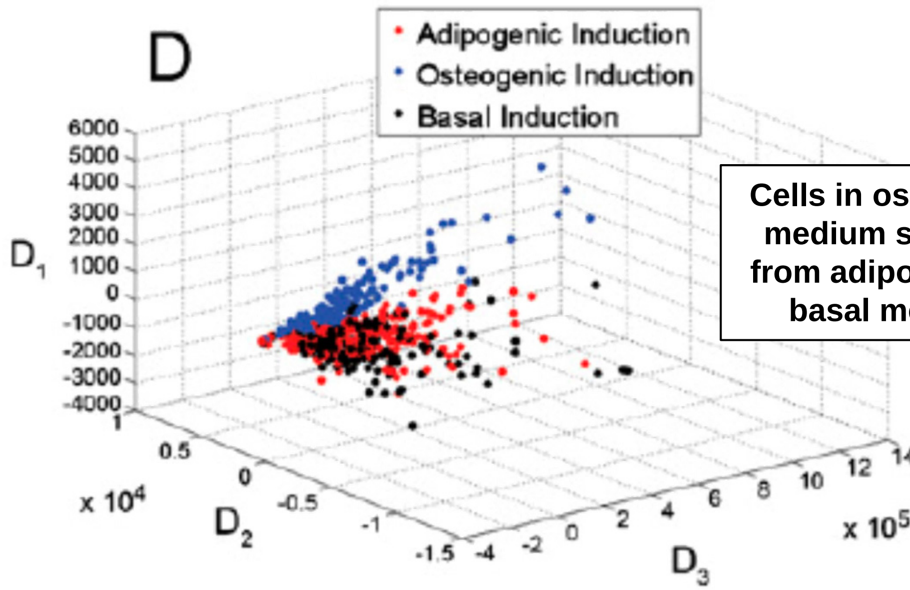
hBMSC Morphology & hBMSC State



Adipogenic Medium | **Basal Medium** | **Osteogenic Medium**

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



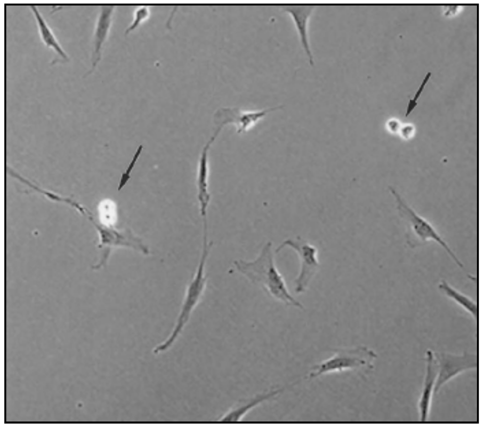
Cells in osteogenic medium segment from adipogenic or basal medium

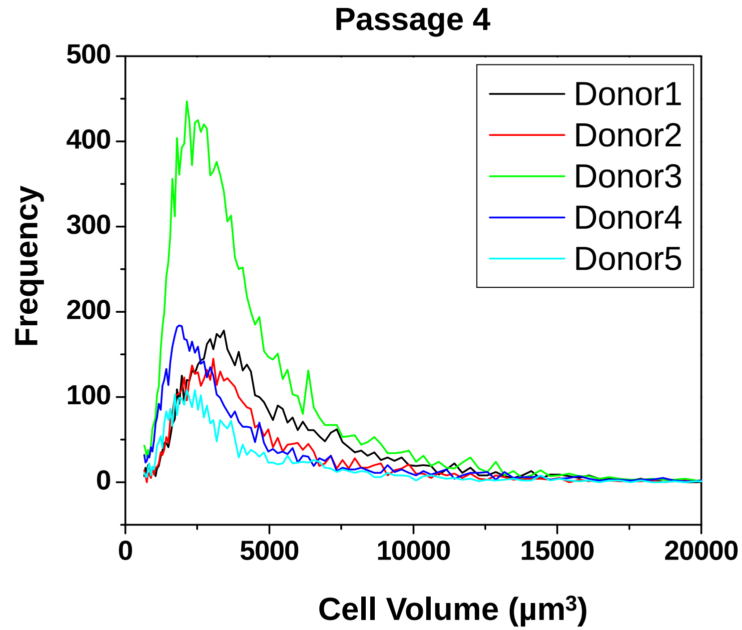
High-content imaging, 43 shape descriptors, condense non-linearly into 3 dimensions and segment

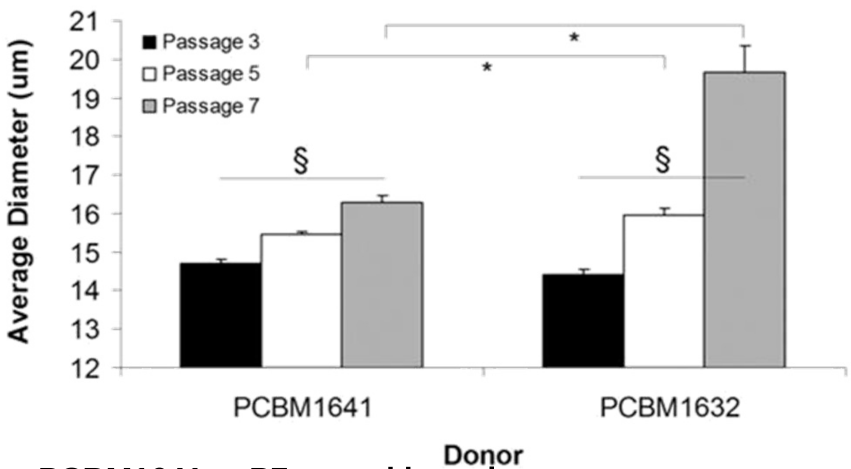
Treiser MD, Yang EH, Gordonov S, Cohen DM, Androulakis IP, Kohn J, Chen CS, Moghe PV. Cytoskeleton-based forecasting of stem cell lineage fates. PNAS 2010;107, 610-5.

Cell Volume: Small hBMSCs More Potent

Colter DC, Sekiya I, Prockop DJ. PNAS v98, p7841, 2001







Donor #	ID #	Sex	Age (Years)	Diameter (Mean ± S.D.) (μm)
Donor 1	7038	Female	29	19.0 ± 0.3
Donor 2	8001	Female	24	18.6 ± 0.2
Donor 3	7071	Male	22	18.1 ± 0.1
Donor 4	7083	Male	24	17.7 ± 0.2
Donor 5	7076	Female	37	17.7 ± 0.0

PCBM1641 at P7 sorted by volume:

- Large = 19.3 μm = 1/296 (+) for adipogenesis
- Small = 14.6 μm = 1/126 (+) for adipogenesis
- $p = 0.02$

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

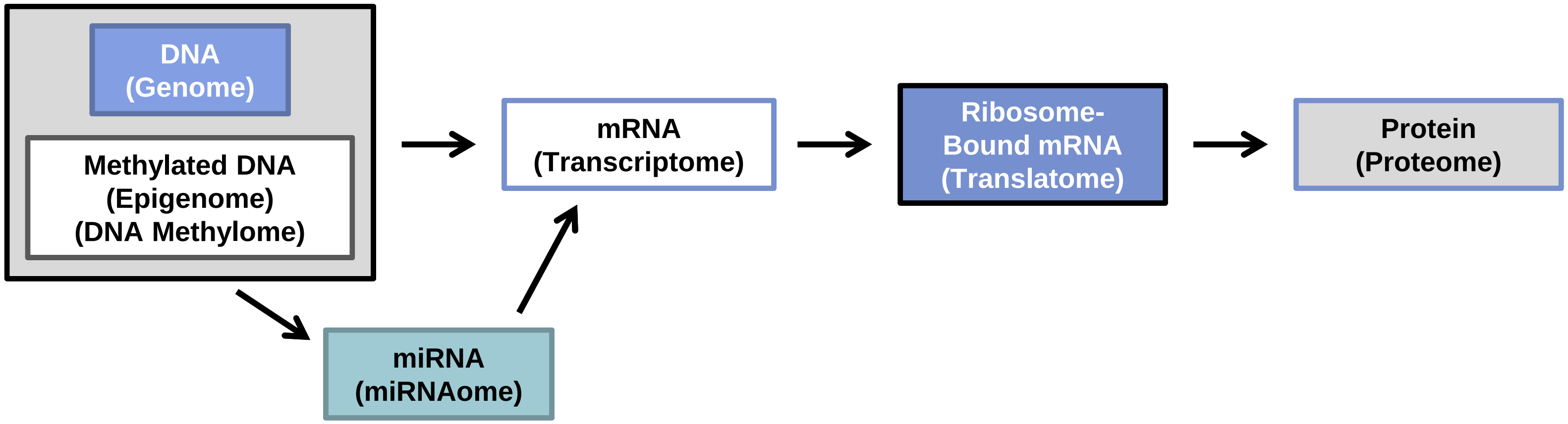
Conclusions & Future Directions

Conclusions

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

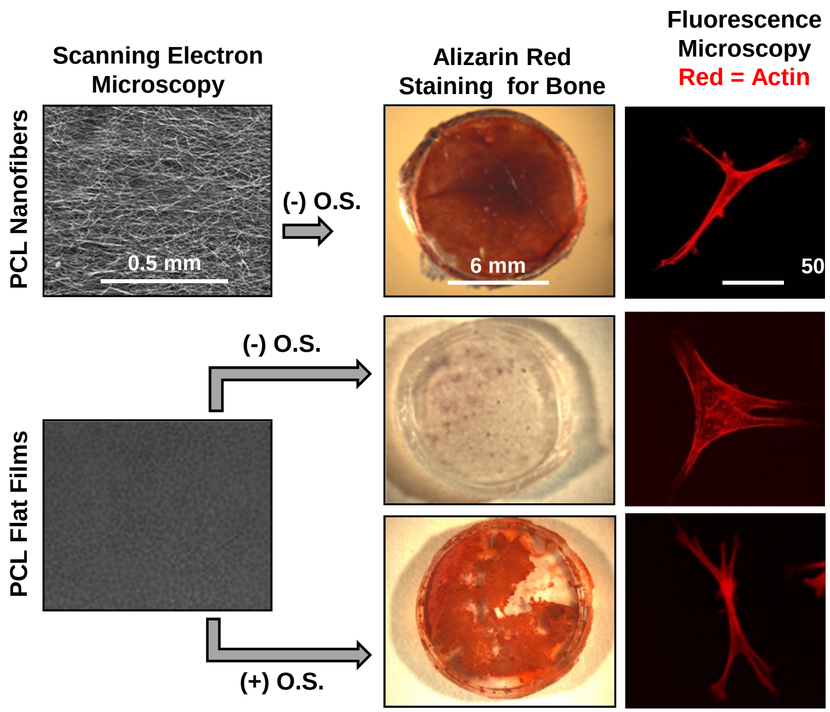
Future:

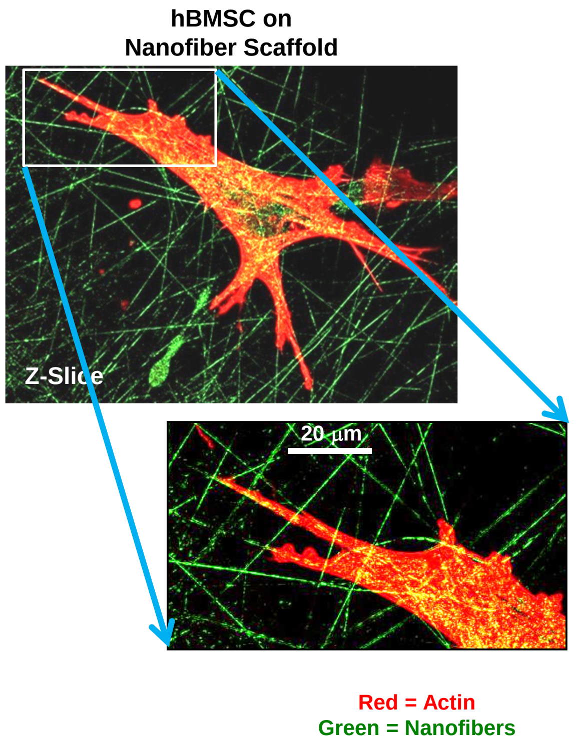
- Proteomics
- Multi-donor microarray experiment
- Cell shape and machine learning
- Cell volume
- Integrome
- Connectome = Integrome + Cell Shape Metrics



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

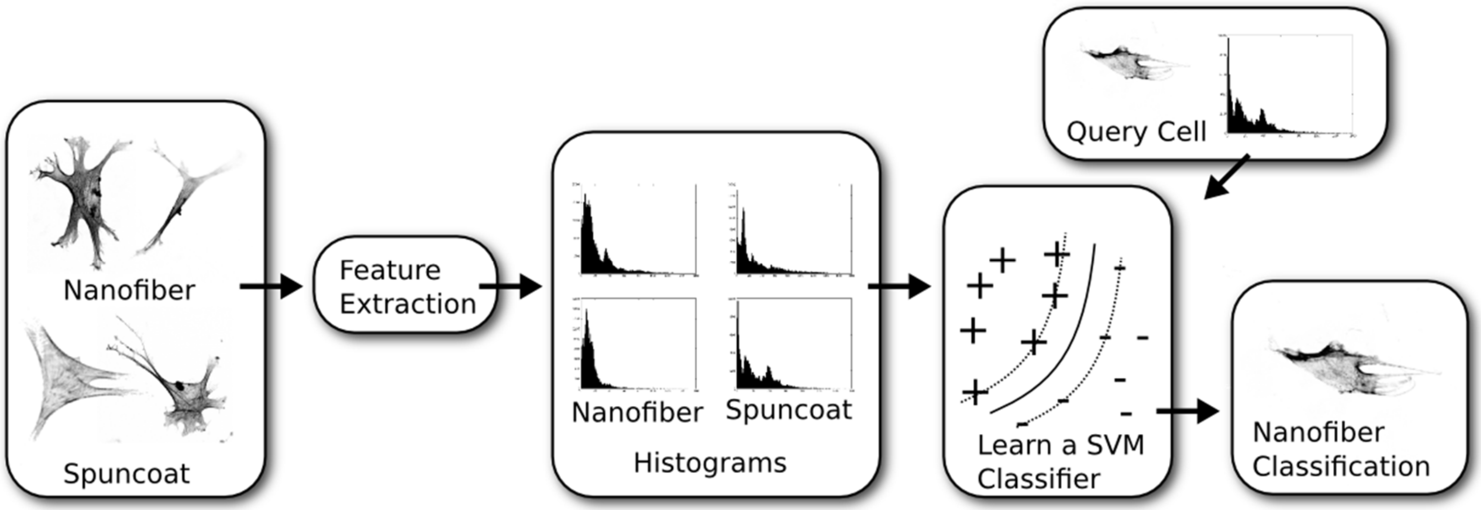
Big-Data-Driven Regenerative Medicine

- High-throughput Imaging
- High-performance Computing and Visualization
- Geometry-driven Quantitative Analysis

Data-driven Classification of Stem Cells

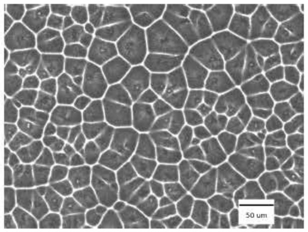
Data Source:

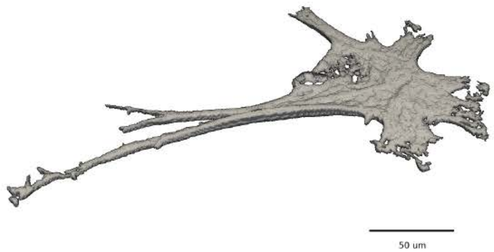
- We used stacks of confocal microscopy images of size 2048 x 2048 x ~20
- Our sample set contained 41 cells, but future drug discovery applications may have 1000s of cells

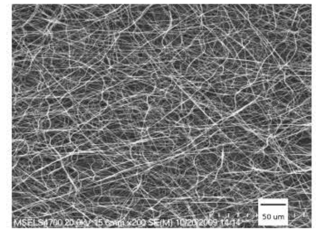


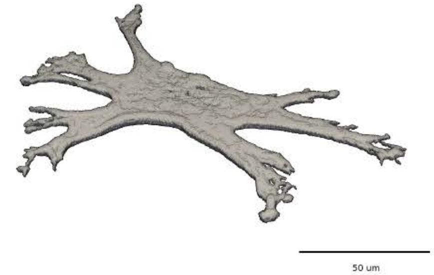
"Parallel Geometric Classification of Stem Cells by Their 3D Morphology". Juba, Cardone, Ip et al. 2013.

Classification of Stem Cells

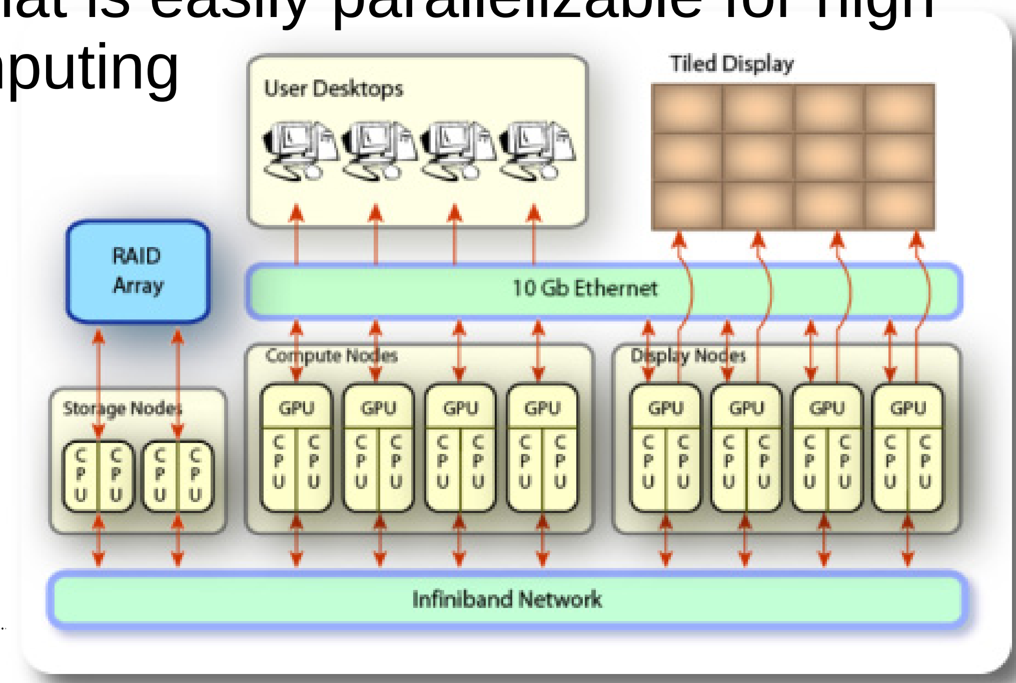






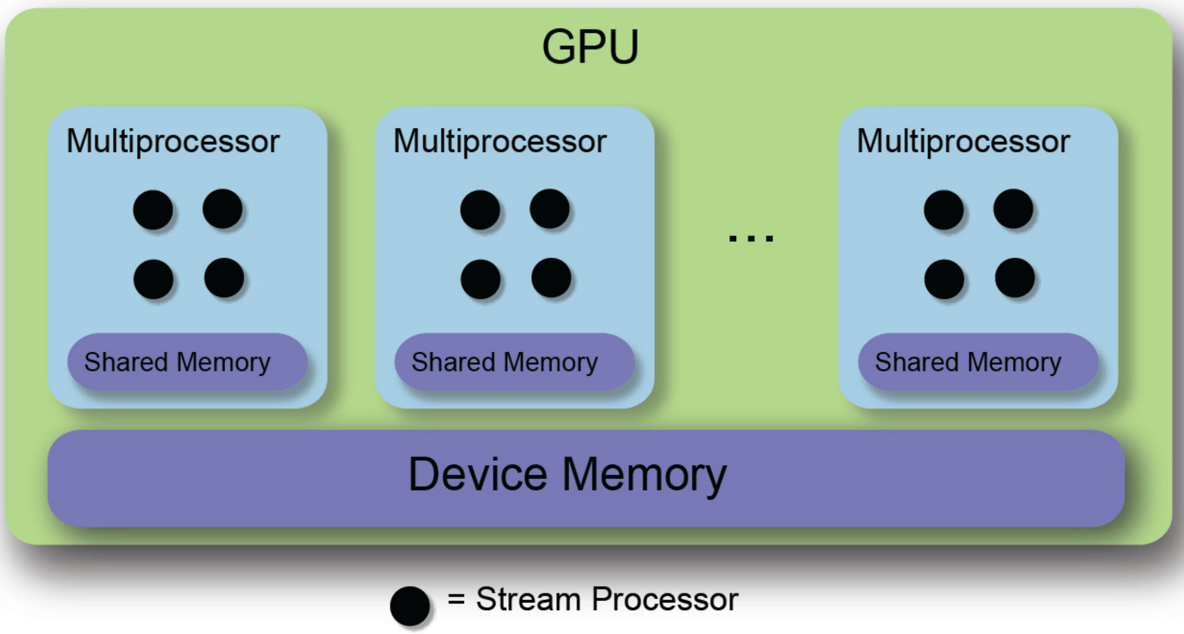


- Cells with more thin branches are expected to have a larger number of short intersections
- Need an algorithm that is easily parallelizable for high throughput data computing

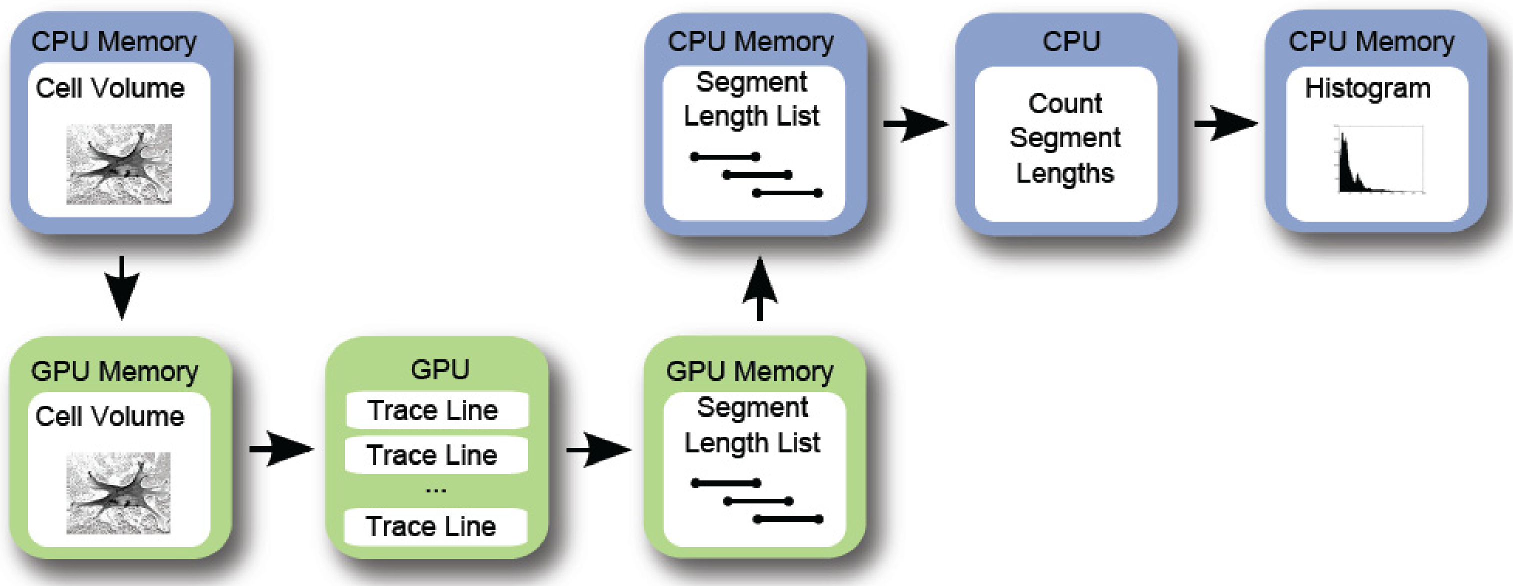


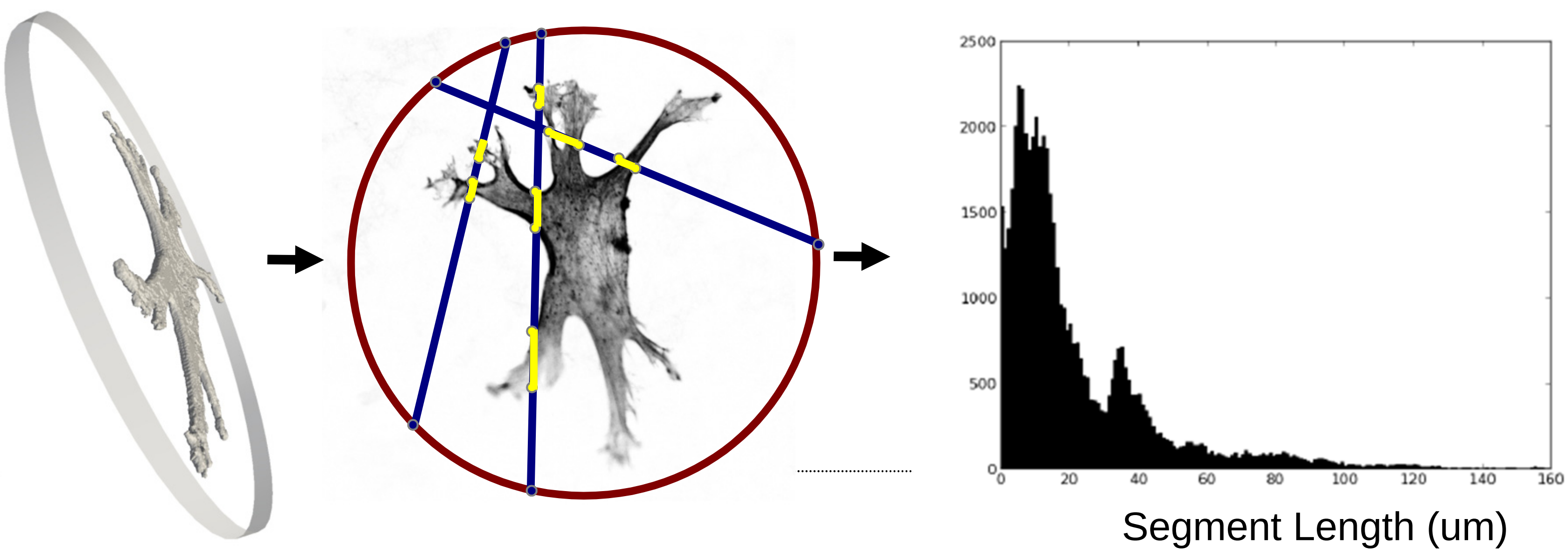
Computing on CPUs and GPUs

- High memory bandwidth
- High number of cores
- High computational capability
- Partitioning the computational task between CPUs and GPUs



In Silico Cell Shape Analysis





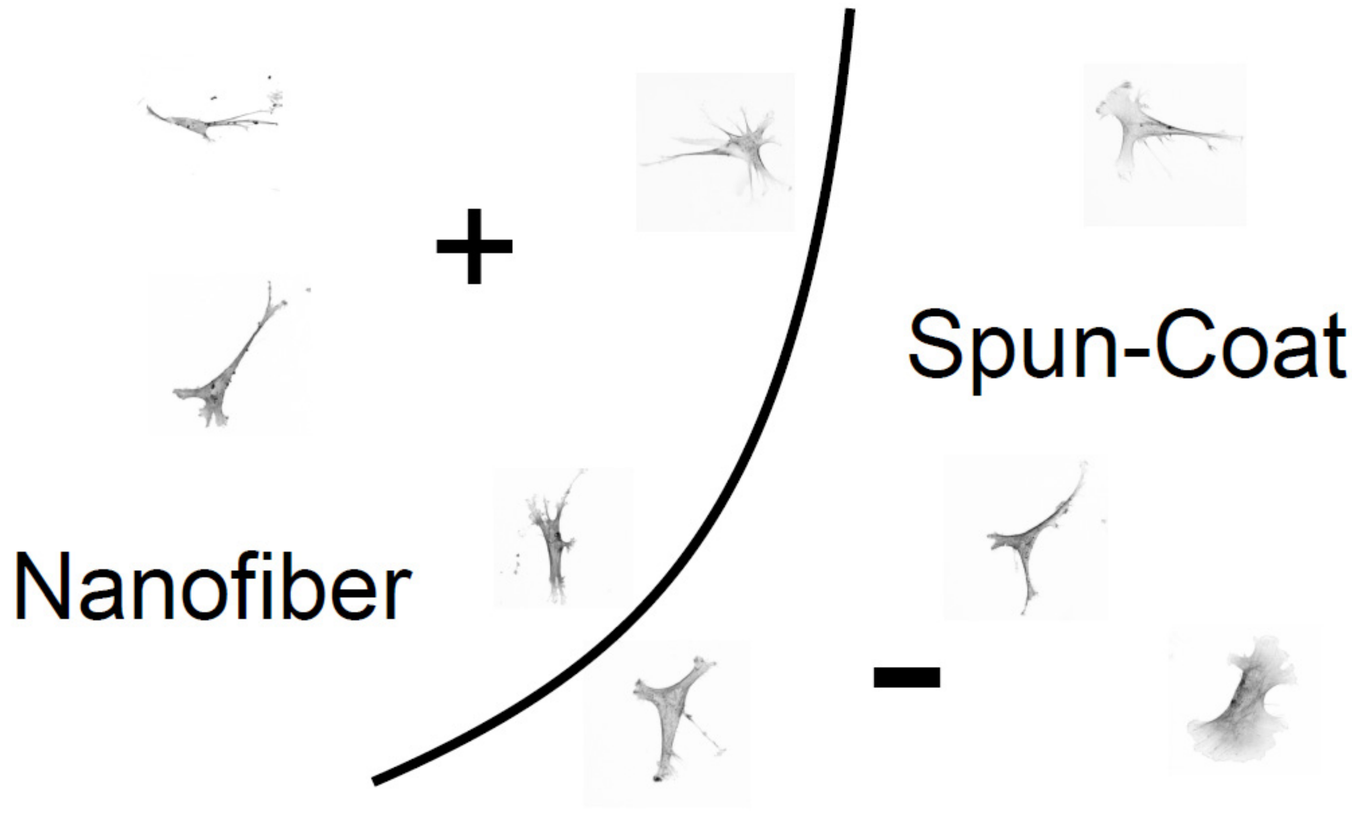
Classification of Stem Cells

Number of Lines	10^3	10^4	10^5	10^6
CPU (ms)	50.1	492	4915	49154
GPU Atomic (ms)	3.56	6.74	45.7	450
GPU Reduction (ms)	14.1	20.1	83.4	743
GPU Lists (ms)	3.52	7.35	52.7	501

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)

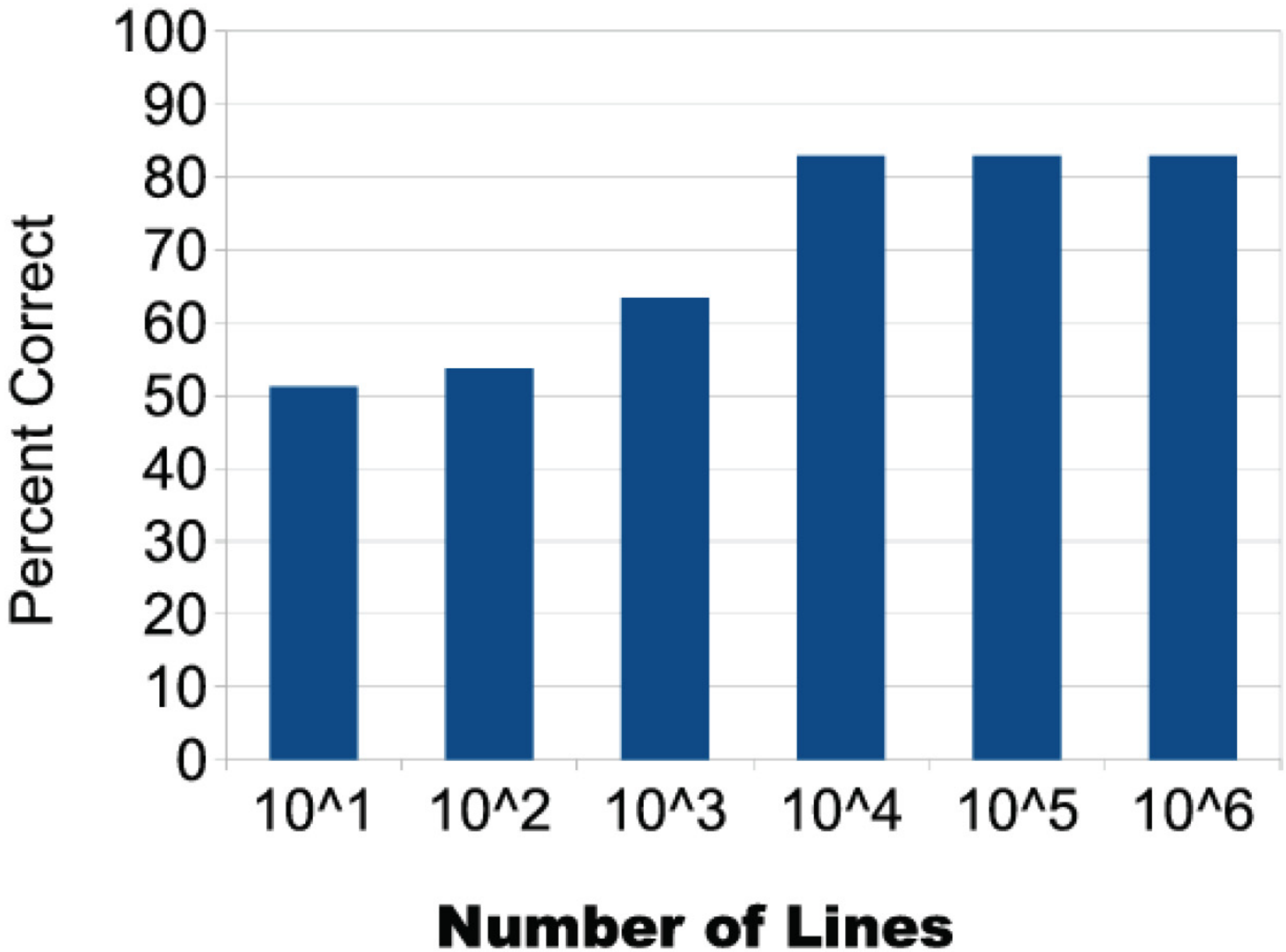
Data-driven Learning

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%



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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