Effect of scaffold structure on 3D shape of stem cells

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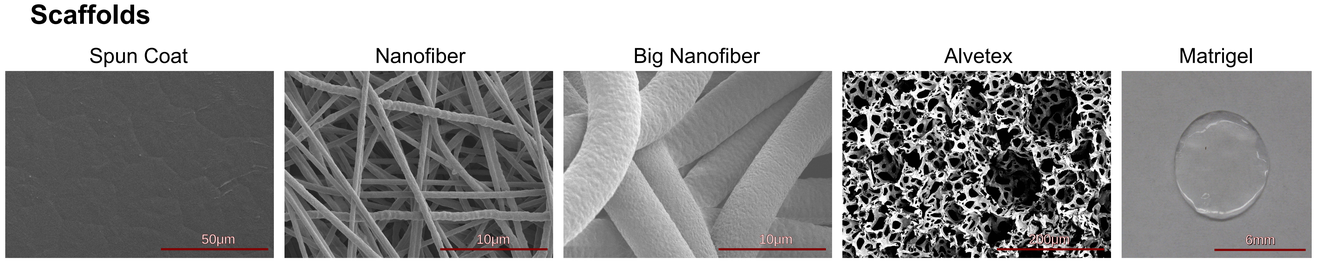
Statement of Purpose: Previous work has demonstrated that stem cell function is influenced by their microenvironment. The chemical, mechanical and structural properties of the stem cell niche can direct stem cell behavior. One mechanism by which the microenvironment influences stem cell function is by modulating stem cell shape. Herein, we have assessed how the 3D shape of primary human bone marrow stromal cells (hBMSCs) is affected by culture on 3D scaffolds with different properties. hBMSCs were cultured on a variety of 3D scaffolds, imaged by 3D laser scanning confocal microscopy, and analyzed using advanced computational algorithms. In order to enhance the rigor of the results, great effort was invested in collecting a large data set, where more than 100 hBMSCs were imaged for each scaffold treatment. Particular focus was placed on analyzing hBMSC dimensionality, in order to assess if 3D scaffolds drove hBMSCs into shapes with greater 3D character. Hundreds of scaffolds with different compositions, pore sizes and mechanical properties have been advanced for tissue engineering applications. The methods developed here provide a tool for classifying these scaffolds based on the stem cell morphologies that they elicit.

Biological Methods:

- PCL nanofibers (NF) were electrospun from solutions of poly(ε-caprolactone)
  - PCL-NF Dia. = 589 nm, S.D. 116, n = 151 fibers
  - PCL-BNF Dia. = 4378 nm, S.D. 419 nm, n = 54 fibers
- For planar controls, PCL was spuncoat (SC) onto TCPS
- Alvetex scaffolds are porous polystyrene (Reinnervate, Inc.)
- Matrigel is a gel formed from extracellular matrix (BD Biosciences)
- Primary human bone marrow stromal cells were seeded onto specimens (hBMSCs, Texas A&M, pass 5, 2500 cells/well in 12-well plates)
- Osteogenic supplements (OS) = 10 nM dexamethasone, 20 mM beta-glycerophosphate, 0.05 mM L-ascorbic acid
- After 24 h culture, samples were fixed & stained
  - Actin = Alexa Fluor 546-phalloidin
  - Nucleus = DAPI
- Z-stacks of fluorescence confocal images were collected on a Leica TCS SP5 laser-scanning confocal microscope
- Voxel dimensions 240 nm × 240 nm × 710 nm
- Actin and nuclei were imaged in >100 hBMSCs per treatment

Sample	Description
Spun Coat N = 119	PCL, planar film
SC + OS N = 114	Spun Coat + osteogenic supplements
Nanofibers N = 114	PCL, ~500 nm diameter fibers
NF + OS N = 113	Nanofibers + osteogenic supplements
Big Nanofiber N = 115	PCL, ~4 μm diameter fibers
Alvetex N = 116	Polystyrene, 200 μm thick, 36 – 40 μm dia pores
Matrigel N = 115	Extract from mouse tumor, mainly Type IV collagen

Scaffolds

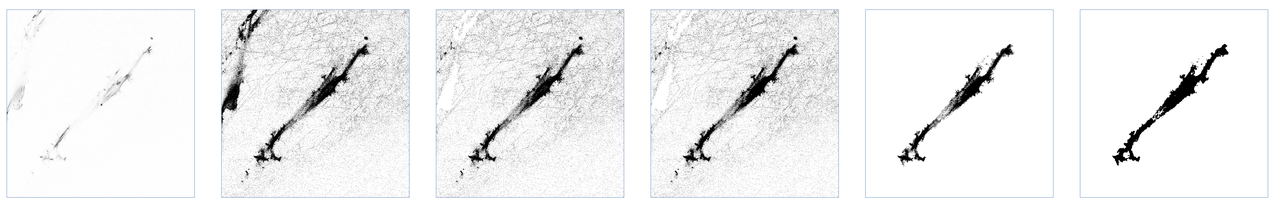


Images of scaffolds. The leftmost four are SEM, the rightmost is a macroscale photograph.

Computational Methods:

- Cell segmentations were computed as follows
  1. Cell data received as 8-bit 3D images
  2. Threshold to mark voxels brighter than the mean as cell
  3. Remove contiguous components touching the edges
  4. Fill completely enclosed voids (in 3D)
  5. Remove all contiguous components except the largest
  6. Apply morphological Close and Open operations to remove divots and bumps
- Volume was computed by counting voxels marked as cell
- Surface Area was computed by counting exposed surfaces of voxels marked as cell
- L1 Depth was computed by projecting all voxels onto the L1 axis and finding the distance between the most extreme projected voxels

Segmentation Steps



1. Original
2. Thresholded
3. Edge components removed
4. Voids filled
5. Non-largest components removed
6. Morphological operations applied

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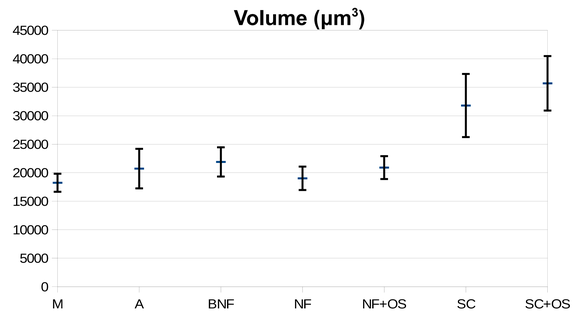
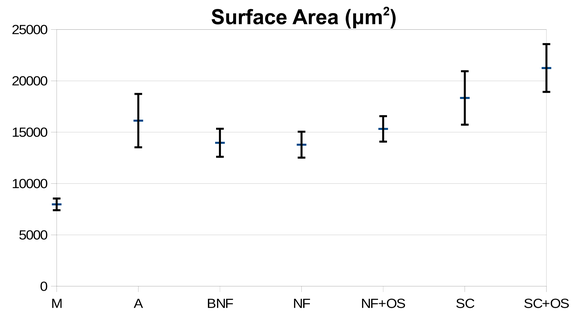
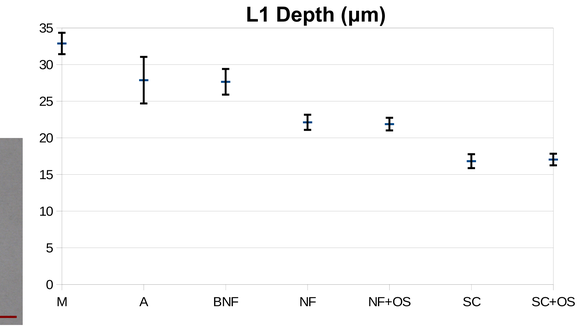


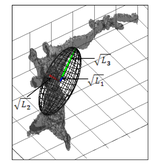


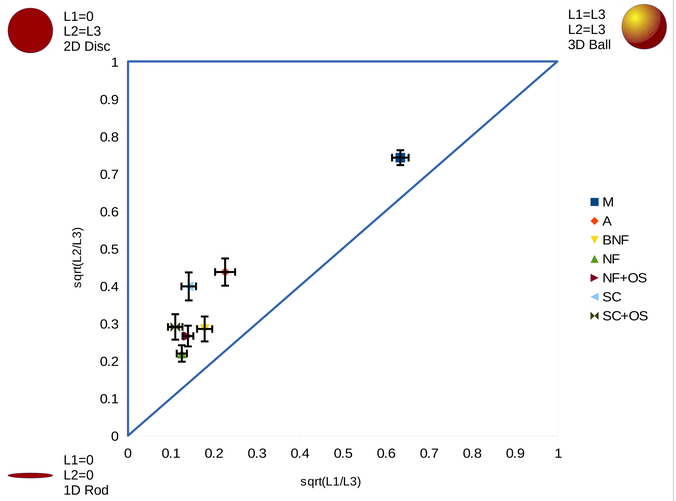


Gyration Tensor:

- Method to fit a characteristic ellipsoid to the 3D cell shape
- The eigenvectors of the gyration tensor, S, are the axes of the ellipsoid and describe cell orientation
- The square roots of the eigenvalues of S are the lengths of the axes and describe cell shape
- We label the eigenvalues (the moments of gyration) as L1 < L2 < L3

$$S = \begin{bmatrix} S_{xx} & S_{xy} & S_{xz} \\ S_{yx} & S_{yy} & S_{yz} \\ S_{zx} & S_{zy} & S_{zz} \end{bmatrix} \qquad S_{ij} = \frac{1}{N}\sum_{k=1}^{N}\left(r_i^k - r_i^{CM}\right)\left(r_j^k - r_j^{CM}\right)$$





Plot of average axis lengths of the characteristic ellipsoid. Lengths have been normalized by L3 to remove the effects of cell size. The position of a scaffold type within the triangle indicates whether its cells are more Rod-like, Disc-like, or Ball-like.

Conclusions:

- We present plots of mean Volume, Surface Area, L1 Depth, and Moments of Gyration, with error bars showing ±2 standard deviations of the mean
- Spun Coat cells tended to have larger Volume and Surface Area than Nanofiber cells, with or without OS
- Nanofiber cells tended to have larger L1 Depth than Spun Coat cells, with or without OS
- Adding OS to either Nanofiber or Spun Coat cells did not tend to change Volume, Surface Area, or L1 Depth
- For the Moments of Gyration, Nanofiber, Nanofiber+OS, and Spun Coat+OS cells were similar to each other
- Spun Coat (without OS) cells were less Rod-like and more Disc-like than Nanofiber, Nanofiber+OS, and Spun Coat+OS cells
- Big Nanofiber cells were similar to regular Nanofiber cells, except that they had a greater L1 Depth
- Matrigel cells were much more Ball-like than the others

Future Work:

- Complete imaging of cells on additional scaffolds
- Examine interaction of nuclear shape with cell shape
- Implement additional computational measurements
- Improve cell segmentation algorithm
- Image and measure the scaffolds themselves

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