Effect of Biomaterial Scaffolds on 3D Shape of Stem Cells

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Motivation:

- Tissue engineering (TE) combines biomaterial scaffolds with cells to regrow tissue.
- An ideal biomaterial scaffold would provide cues to put human bone marrow stromal cells (hBMSCs) in desired 3D cell niche for the TE application.
- There is a variety of biomaterial scaffold structures, however there is a lack of characterization of the 3D cell niche.
- hBMSCs are a heterogeneous cell population and there is little quantitative 3D cell shape analysis in response to biomaterial substrates.
- Surveying a variety of biomaterial scaffolds would provide information about the cell shape promoted by each scaffold.

Purpose:

- Quantitatively evaluate 3D cell shape on biomaterial substrates.
- Collect large dataset to characterize heterogeneous hBMSC population with greater statistical rigor.
- Classify substrates by cell niche (1D, 2D, or 3D).
- ITL team provided computational tools for segmentation and data analysis.

Abbrev	Description	Properties [mean (S.D.)]	# Cells Imaged
SC	Flat Spuncoat Films of PCL*	Surface Roughness = 92.76 nm (10.69 nm)	99
SC+OS	Flat Spuncoat Films of PCL with OS*	Surface Roughness = 92.76 nm (10.69 nm)	96
NF	Electrospun PCL Nanofibers	Fiber Dia. 589 nm (116 nm)	101
NF+OS	Electrospun PCL Nanofibers with OS	Fiber Dia. 589 nm (116 nm)	95
MF	Electrospun PCL Microfibers	Fiber Dia. 4.38 µm (0.42 µm)	87
PPS	Porous Polystyrene Scaffold (Alvetex)	Pore Size 36 µm to 40 µm	98
MG	Matrigel	Mouse tumor extract, rich in Type IV collagen	98
FG	Fibrin Gel	Polymerized fibrinogen (6 mg/mL)	92
CG	Collagen Gel	Type I collagen (2.4 mg/mL)	101
CF	Collagen Fibrils	Type I collagen (300 µg/mL), fibril dia. < 1 µm	102

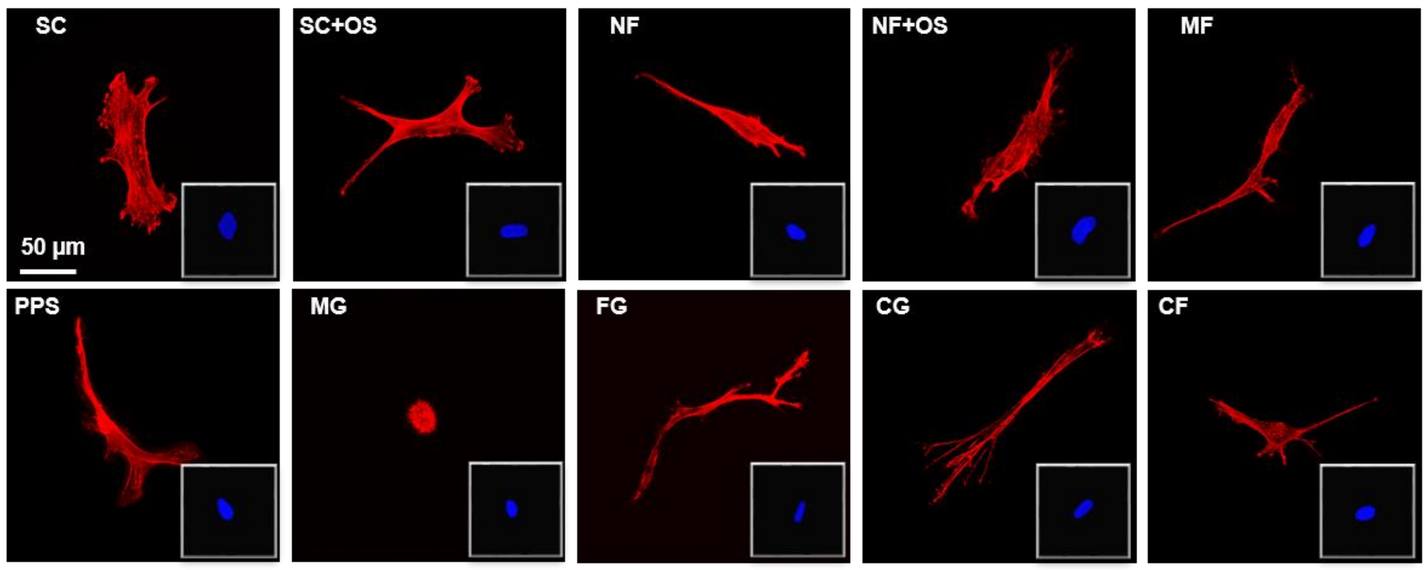
*PCL = Poly(ε–Caprolactone); OS = Osteogenic Supplements

Questions addressed by sample set:

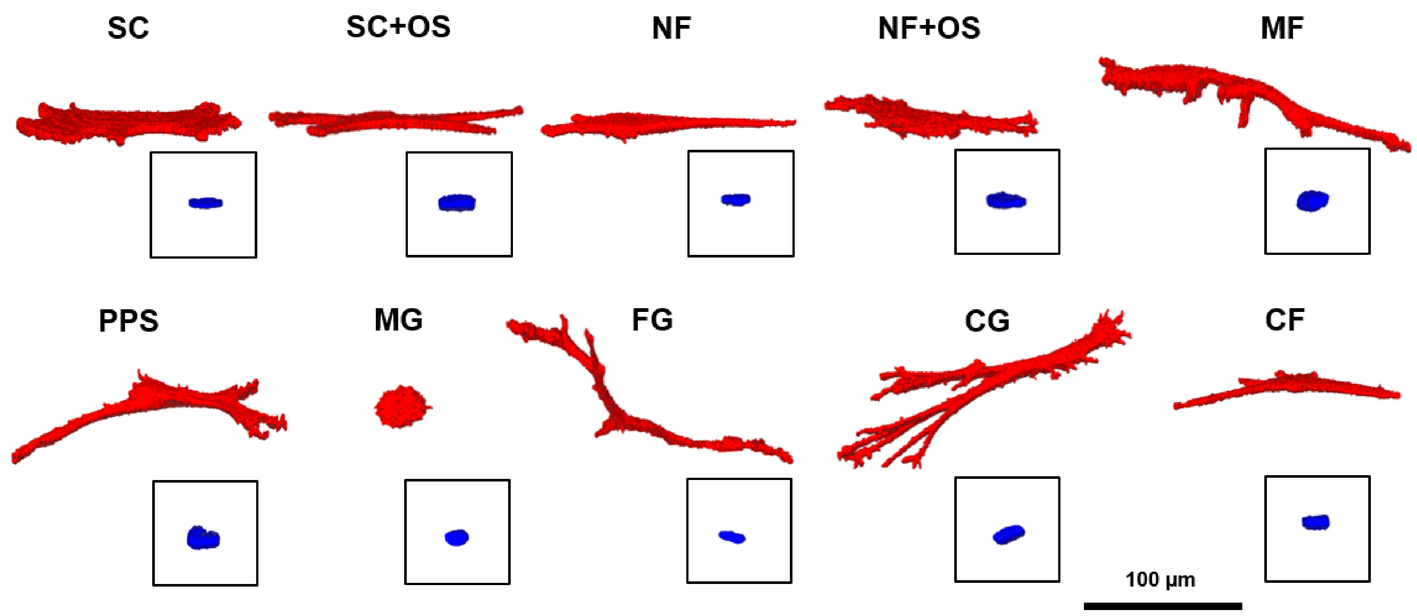
- What are the differences in cell shape between 2D and 3D substrates?
- How does the cell niche change by substrate morphology?
- What is the effect of osteogenic supplements (OS) on cell shape?

Representative Cell Shapes

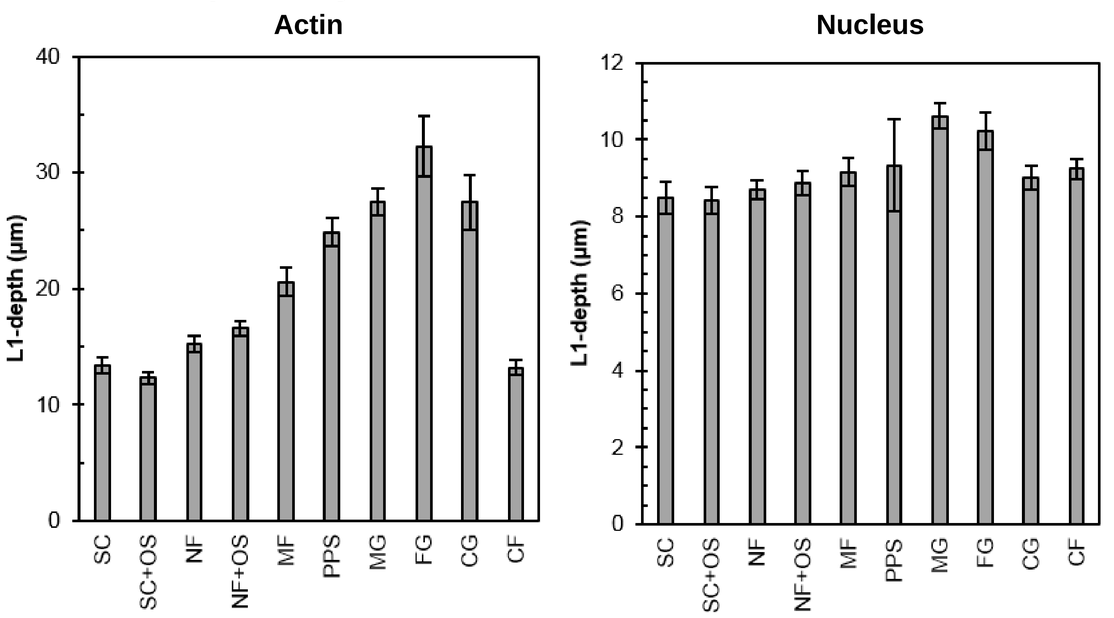
2D maximum intensity projections (xy plane)



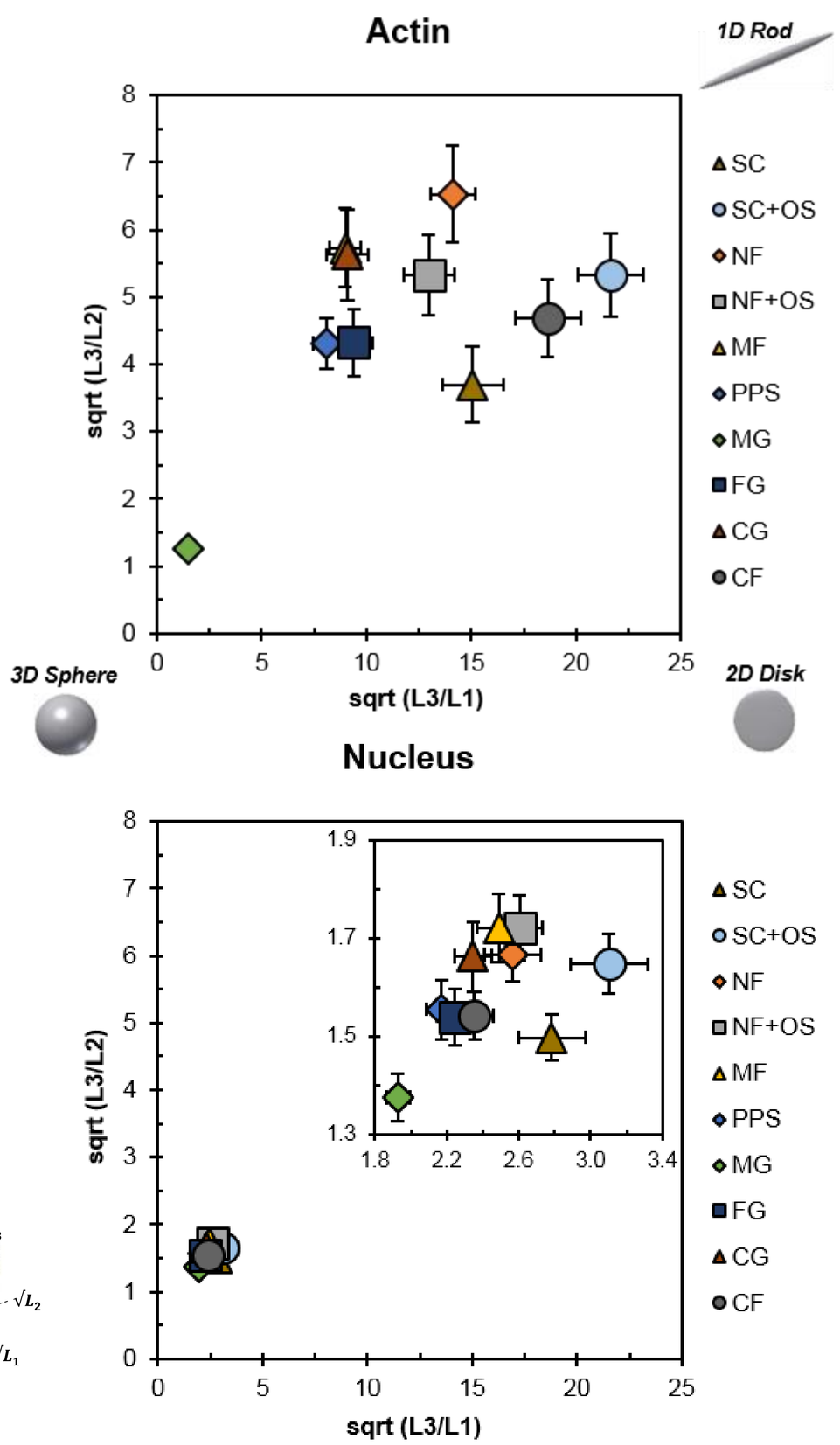
3D rendered images of representative cells

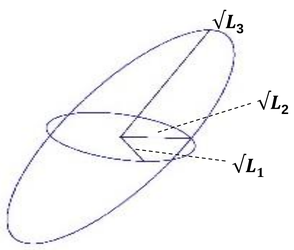


3D Cell Shape Analysis



Bar graphs indicate L1-depth (height) of cells, with actin cytoskeleton (right) and nucleus (left) measured independently, n > 85 cells per group. The error bars denote 2 standard deviations of the mean.





Figures indicate ratio of gyration moments for actin cytoskeleton (top) and nucleus (bottom) for each sample group. The dimensionality matrix has 1D, 2D, or 3D cell shape regions. The inset on nucleus figure shows a close up of the region at the lower left to provide greater detail. The image at lower left shows how the gyration moments are defined.

Conclusions:

- Actin L1-depth (height) shows considerable differences by biomaterial substrate, while nucleus L1-depth does not have a strong trend.
- Cell shape can be divided into 1D, 2D, and 3D regimes based on gyration moment ratios.
  1D – NF 2D – SC 3D – MG
- Nuclear shape has a weak correlation with cell shape based on ratios of gyration moments.
- 3D confocal imaging needs better validation to quantify measurement uncertainties.
- Dataset provides a means to classify substrates by cell niche and provides the TE community information about desirable substrates to promote different 3D cell shapes.

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