3D Stem Cell Morphotyping of Tissue Engineering Scaffold Niches

Stephen J. Florczyk[1], Mylene Simon[2], Derek Juba[2], Sumona Sarkar[1], Desu Chen[1], Paula J. Baker[1], Antonio Cardone[2], Mary Brady[2], Peter Bajcsy[2], Carl G. Simon Jr.[1]

[1]Biosystems and Biomaterials Division, National Institute of Standards and Technology, Gaithersburg, MD

[2]Software and Systems Division, National Institute of Standards and Technology, Gaithersburg, MD

Statement of Purpose: Researchers conduct studies using 3D biomaterial scaffolds but do not typically characterize cell shape. There is currently no method for assessing the nature of the niche provided by 3D culture platforms. Analyzing human bone marrow stromal cell (hBMSC) 3D cell shape in response to different biomaterial scaffolds allows the 3D cell niche promoted by biomaterial scaffolds to be evaluated. 3D cell shape is an important parameter to characterize the effects of scaffold microenvironment on stem cell response.

Methods: Primary hBMSCs were obtained from Tulane University and expanded in growth media until passage five then seeded (5,000 cells/cm^2) in biomaterial scaffolds and cultured for 24 h in standard culture conditions. The biomaterial scaffolds investigated include: poly(ε-caprolactone) (PCL) spuncoat film (SC); PCL SC with osteogenic supplements (OS), (SC+OS); PCL nanofibers (NF); PCL NF with OS (NF+OS); PCL microfibers (MF); porous polystyrene scaffold (PPS); Matrigel (MG); fibrin gel (FG); collagen gel (CG); and collagen fibrils (CF). Samples were fixed and stained for actin and nucleus, imaged with confocal microscopy to obtain a 3D volume (z-stack), and 3D cell shape was analyzed with computational approaches. Over 100 cells were imaged per scaffold group (10 scaffold groups, ~1250 cells total), resulting in the largest known 3D stem cell dataset (~135,000 files, ~135 GB) and enabling a high degree of statistical rigor to be achieved. The images were segmented using a new automated algorithm that was verified by manual segmentations. A final dataset of 969 cells that were well segmented for actin and nucleus were prepared and analyzed with 79 shape metrics that provided 2D and 3D comparisons. The shape metrics enabled 3D stem cell morphotyping of scaffold niches.

Results: The variety of scaffolds studied promoted different cell morphologies during culture. Representative cells for the scaffold groups as rendered in our online 3D cell viewer are shown in Fig. 1. There were significant differences in shape metrics for hBMSCs cultured on the biomaterial scaffolds, particularly for cell depth, surface area, and volume. Addition of osteogenic supplements to nanofiber and spuncoat samples did not strongly influence cell shape from samples without supplements. Comparison of three hydrogels, Matrigel, collagen gel, and fibrin gel, produced different cell shapes, where the Matrigel promoted a 3D spherical shape, while the collagen gel and fibrin gel promoted a 1D, predominantly linear shape. Analysis of nuclear metrics did not show as strong of differences as actin. Comparison of cell shape metrics with morphotyping indicated variant and invariant parameters influencing cell shape.

Conclusions: This study demonstrated a quantitative approach to analyze 3D cell shape and morphotype and is the largest known study analyzing 3D cell shape in response to a variety of biomaterial scaffolds. Multi-parametric analysis over a wide range of scaffold types defined the 3D morphotype of cells in the biomaterial environment. The dataset is publically accessible with an online 3D viewer. These results could inform the selection of prospective scaffolds for applications based on 3D cell shape in the native tissue of interest.

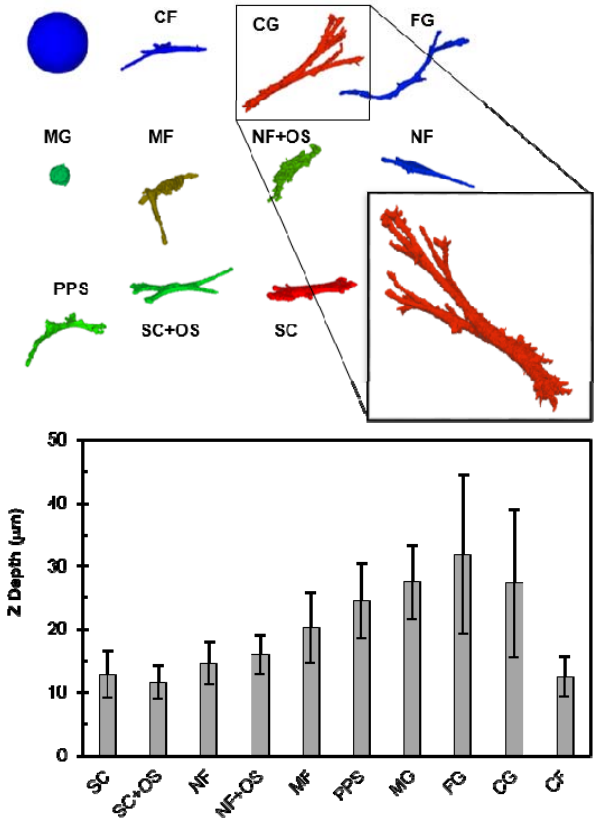

Figure 1. Top: Image from 3D cell viewer of representative cells for 10 scaffolds, color coded by volume (blue is smallest, green is median, and red is largest); blue sphere is 100 µm. Inset indicates how cells may be examined at higher resolution. Bottom: Bar graph of z-depth for the scaffold groups is presented (mean +/- 2 S.D. of the mean).

Acknowledgments: SJF is supported by NIST NRC Postdoctoral Research Associateship.

Disclaimer: This work, a contribution of NIST, is not subject to copyright in the United States. Certain equipment, instruments or materials are identified in order to adequately specify the experimental details. Such identification does not imply recommendation by NIST nor does it imply the materials are necessarily the best available for the purpose.