QUANTIFICATION OF 3D MATRIX DEFORMATIONS INDUCED BY ANGIOGENIC SPROUTS IN FIBRILLAR GELS

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Introduction:

Cell-matrix mechanical interactions play a key role in a variety of physiological processes. Recently, the quantification of cellular tractions by means of traction force microscopy has been extended to 3D cell cultures. However, typically assumed simplifications during the mechanical characterization of the matrix could affect the retrieved traction magnitudes. On the other hand, cell induced matrix displacements already provide quantitative information on cell-matrix mechanical interaction, avoiding the complexity inherent to traction reconstruction. In this study, we assess the deformations induced by angiogenic cellular sprouts in a fibrillar matrix under chemically defined culture conditions from the full field displacements obtained with and without fluorescent beads acting as fiducial markers.

Materials and Methods:

Human umbilical vein endothelial cells (HUVEC) were seeded on top of a collagen gel and induced with pro-angiogenic factors to form multicellular sprouts. The sprouts were grown in endothelial cell growth medium (EGM2) supplemented with or without blebbistatin or cytochalasin D. As control for the calculation of the matrix deformations, 200 nm fluorescent beads were attached to the collagen fibers. Second harmonic generation (SHG) and laser-scanning confocal microscopy were used to acquire Z-stacks of label-free collagen fibers and fluorescent beads, respectively, during the live imaging before and after chemically induced relaxation of cells. The calculation of the matrix deformations was formulated as a B-spline –based 3D non-rigid image registration process that warps the image of the stressed gel to match the image of the gel after relaxation [1]. The calculation of these displacements was independently performed from fiber (without fiducial markers) and bead images.

Results:

We observed that the recovered displacements (Figure 1) from the label-free fiber images were equivalent to the ones obtained from bead images, showing magnitudes ranging between 1 to 8 µm before the blocking effects of blebbistatin or cytochalasin D on acto-myosin force generation.

Conclusion:

Our methodology allows mapping cell-induced 3D matrix deformations around multicellular sprouts embedded in fibrillar gels without the need for fluorescent beads, which could alter the matrix mechanical properties. The resulting information is expected to provide a quantitative view of the cell-matrix mechanical interaction of HUVECs in 3D and leads to a better comprehension of cell mechanobiology in sprouting angiogenesis.

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[1] Jorge-Peñas A et al. (2015) Free Form Deformation–Based Image Registration Improves Accuracy of Traction Force Microscopy. PLoS ONE 10(12): e0144184.

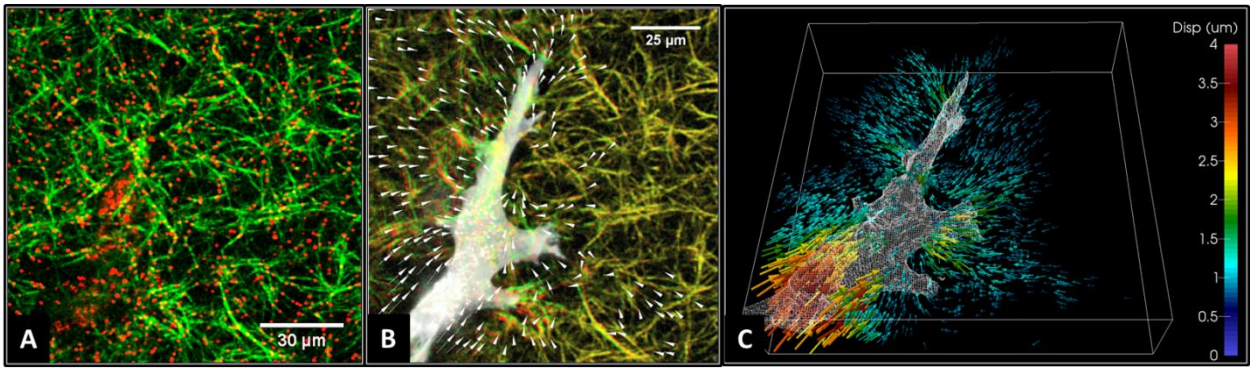

Figure 1: Imaged collagen fibers with attached fluorescent beads (A) and displacement directions (arrows) of collagen fibers (green=stressed, red=relaxed) around HUVEC tip cell (white) (B) for a selected XY plane. Computed 3D full field displacements (in µm) induced by the sprout (C).