NOVEL AND ACCESSIBLE 3D TRACTION FORCE MICROSCOPY APPLIED TO AN IN VITRO VASCULAR DISEASE MODEL

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Introduction

Quantification of active cellular forces is crucial to understand the role of mechanotransduction in modulating cell behavior. Traction force microscopy (TFM) is well accepted for computing cellular forces in 2D cell cultures, but its application to 3D cultures remains challenging[1,2]. Questions on 3D TFM accuracy remain partly unresolved. Moreover, more accessible, easy-to-use 3D TFM workflows that can be applied to biologically relevant in vitro models are needed. In this work, we developed a novel 3D TFM method that was integrated into an image-based workflow for analysing the role of cellular forces in angiogenesis. This method was then applied in a specific vascular disease context, namely Cerebral Cavernous Malformation (CCM), a genetic disease related to mutations of CCM genes and characterized by microvascular lesions in the brain, similar to those encountered in tumor blood vessels. This method was used to reveal differences in 3D cellular tractions between healthy (control) and diseased (CCM-2 depleted) human umbilical vein endothelial (HUVEC) cells during vascular invasion.

Methods

We adapted an in vitro sprouting angiogenesis model to make it compatible with 3D TFM[3]. HUVECs (control vs CCM-2 depleted) were cultured and allowed to invade a synthetic modular polyethylene glycol (PEG) matrix containing 200 nm fluorescent beads for live imaging of matrix deformations during invasion. Image stacks at different time points were acquired by means of confocal image microscopy and matrix deformations were accurately computed by means of free form deformation-based image registration (FFD)[4–6]. Cellular tractions were then inferred from these deformation fields and measured PEG elastic properties by means of our novel physics-based nonlinear inverse method (PBNIM) which constrains the displacement field to a physically consistent solution. PBNIM was also validated by generating ground truth displacement and traction fields using the geometry of a real angiogenic sprout and simulating force exertion through focal adhesions. We compared PBNIM to a standard forward traction recovery method.

Results

Our proposed inverse method (PBNIM) outperformed a standard forward traction recovery method showing an enhanced accuracy (average error of 17±12.2% vs 31.5±27.3%) and better capturing ground truth sparse traction patterns (see Figure 1a). Moreover, when analyzing real experimental data from control versus CCM-2 depleted HUVECs, our 3D TFM workflow revealed pulling traction maxima near the sprout tips (see Figure 1b) that were higher upon CCM-2 loss.

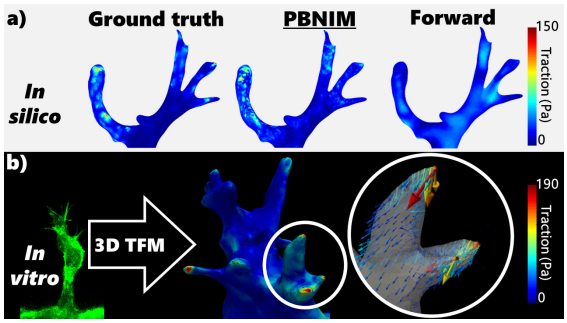

Figure 1: (a) Validation of our traction recovery method (PBNIM). A simulated ground truth solution is compared to PBNIM and a standard forward method using an angiogenic sprout geometry. (b) Confocal microscopy image of an angiogenic sprout invading a PEG hydrogel (left), and traction forces recovered by our 3D TFM workflow (right).

Discussion

In this work, we developed a novel 3D TFM method, proved its accuracy by means of realistic ground truth simulations and integrated it into an image-based workflow that can be easily used. Its usefulness as a screening tool for improved disease modelling was demonstrated by revealing differences in cellular tractions during sprouting angiogenesis between healthy and diseased conditions.

References

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