3D Microfabricated scaffolds for the investigation of the mechanical forces exerted by living cells during migration

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Mechanical forces play a pivotal role in many cellular processes, such as migration. The molecular mechanisms and nano-scale machineries involved in cell force generation are well characterized,[1] but the regulation of their activities at the whole cell level during a physiological process is far from being understood. We propose a methodology that could help elucidate this complex phenomenon. The basic idea is to fabricate a 3D scaffold – at the cellular scale –composed of deformable beams that can be bent by living cells migrating inside it. By recording a video sequence of the scaffold deformations we can learn how the mechanical activity of the cell is regulated in space and time during migration. In this work, we present a simple fabrication method based on 2-photon optical lithography, and show the functionality of the produced scaffolds by investigating the migration of human macrophages. Macrophages play beneficial roles in protective immunity; however, when they infiltrate diseased tissues, they also favor the progression of a range of pathologies, including chronic inflammation, cancer and obesity.[2] Therefore, it is a challenging issue to investigate the mechanisms involved in their migration. Macrophage migration has mostly been studied in 2D systems that do not involve the distinct environmental constrains of natural 3D microenvironments. Therefore, the scaffolds were designed with dimensions such that a macrophage entering one of them is spatially confined and senses a relevant 3D environment.

Our results show that macrophages are capable of penetrating resist scaffolds of cubic geometry (Fig. 1) exhibiting a lattice parameter larger than 5 µm and that the scaffold support itself can be used as a sensor to measure the forces exerted by the migrating cells (Figs. 2 and 3). As a typical example, Figure 4 shows that beam displacements as large as 0.3 µm can be recorded during a migration sequence for a 10 µm lattice parameter and 1.5 µm beam diameter. Through the measurement of the stiffness of the resist material and by solving the equation of elasticity using COMSOL software, we will show that the cellular force of a migrating macrophage in a 3D microenvironment can be estimated.

Altogether, our results demonstrate the functionality of the printed scaffolds in understanding the mechanical activity of a living cell in a confined 3D microenvironment over time and space.

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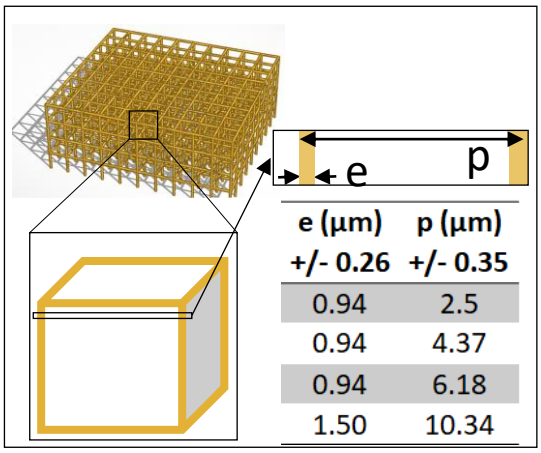
[1] Bouissou, A., Dupuis, G., Fort, E., Lévêque-Fort, S., Maridonneau-Parini, I. and Poincloux, R. (2017). Podosome Force Generation Machinery: A Local Balance between Protrusion at the Core and Traction at the Ring. *ACS Nano,* 11(4), pp.4028-4040

[2] Qian BZ. and Pollard JW (2010). Macrophage diversity enhances tumor progression and metastasis. *Cell,* 141,pp. 39-51



e (µm) +/- 0.26	p (µm) +/- 0.35
0.94	2.5
0.94	4.37
0.94	6.18
1.50	10.34

Figure 1. Design of the cubic scaffolds used in this work, e is the beam diameter and p the lattice parameter.

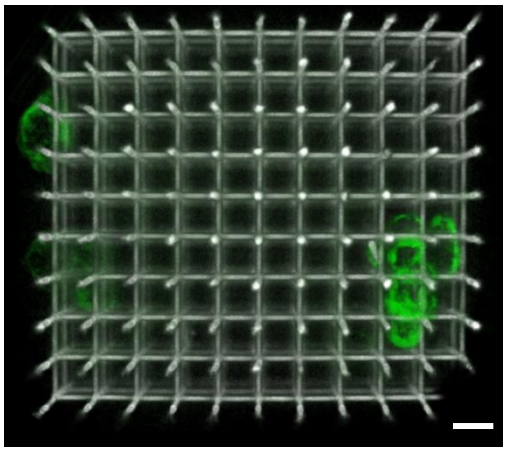


Figure 2. Fluorescent confocal image of 3D scaffold (grey) with macrophages (green) migrating inside. Scale bar 10 µm.

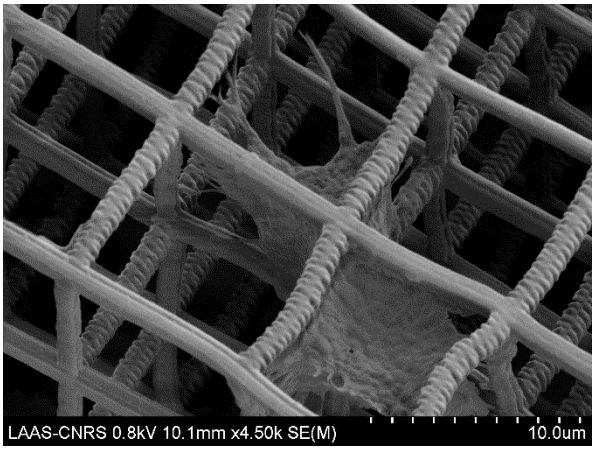


Figure 3. SEM image of a scaffold (p=10 µm) with an embedded human macrophage.

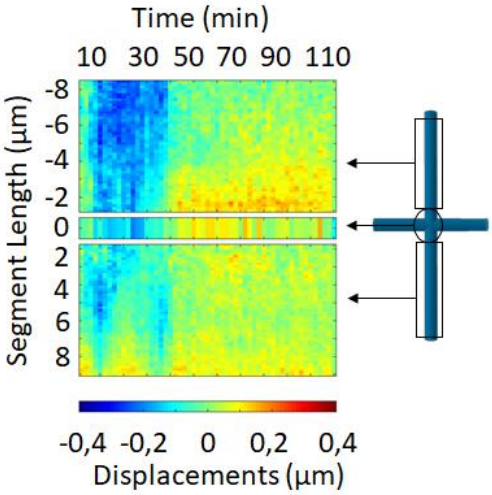


Figure 4. Kymographs of the temporal displacements of two beams and a connecting node in contact with the cell.