Studying nanoscale heterogeneities and dynamics of novel biomimetic polymer gels using advanced microscopy

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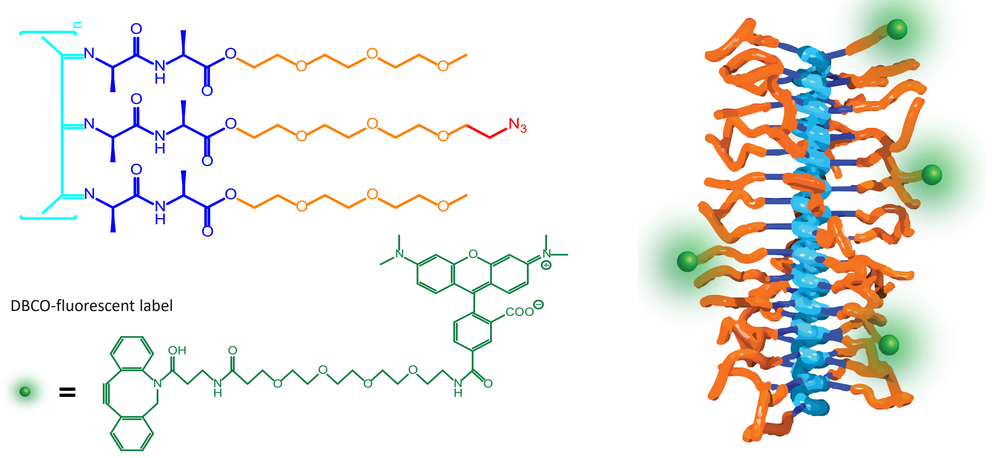
Polyisocyanopeptide hydrogel

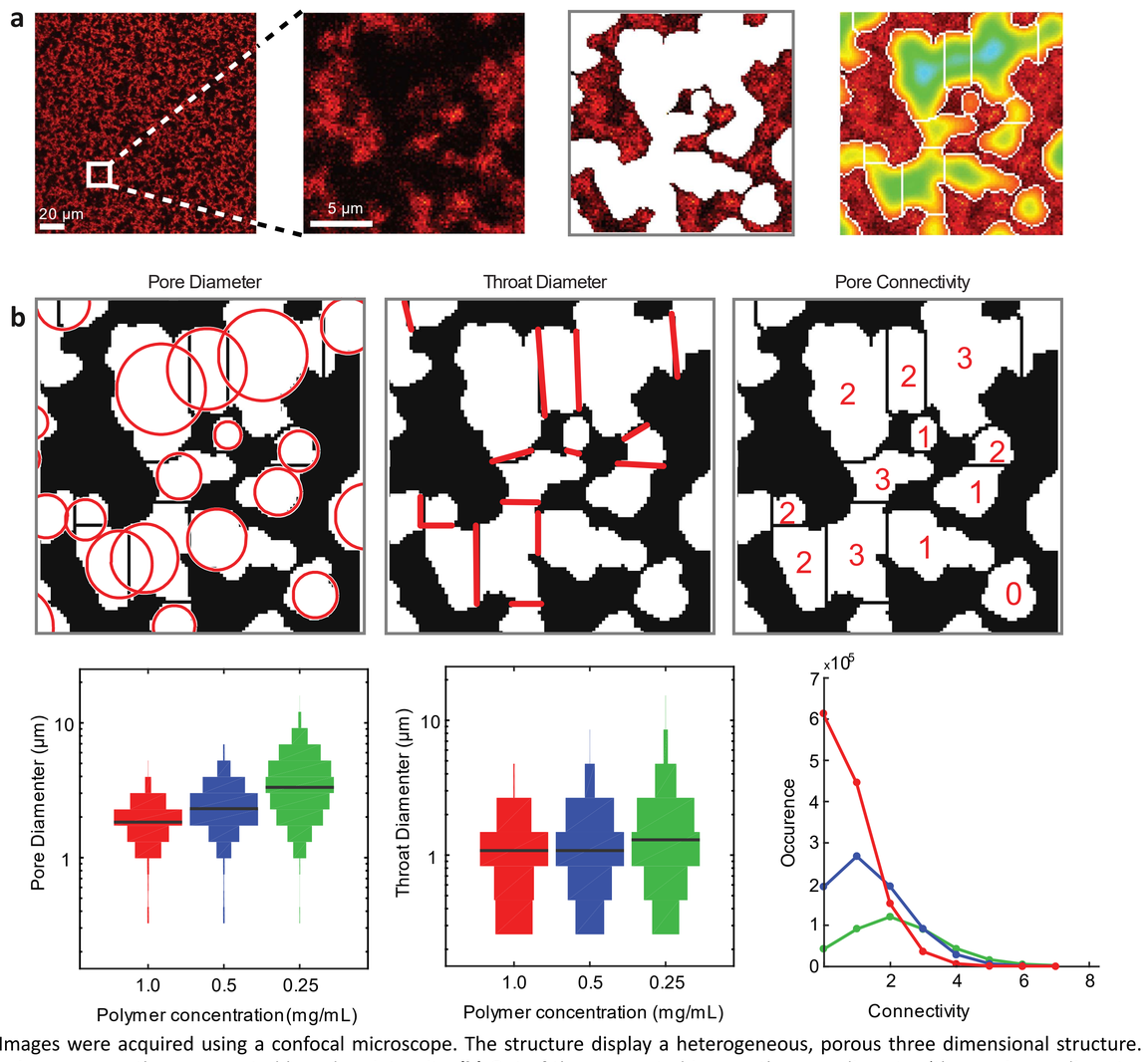
In living tissues, cells are surrounded by a meshwork of proteins and glycosaminoglycans collectively referred to as the extracellular matrix (ECM). A growing interest to understand the influence of the biochemical and mechanical properties of the ECM in cellular behavior has led to the development of new polymer based scaffolds for cell culture. Due to the important role of the mechanical properties in guiding cells, chemists created a new generation of materials with a viscoelastic behavior that closely mimics biopolymer networks. Here we present the structural characterization of hydrogels based on polyisocyanopeptides (PIC) grafted with polyethylene glycol side chains. Previously it was shown that the mechanics of these fully synthetic hydrogels can be tuned by changing the length of the PIC polymer chain or the composition of the side chains [1]. Combined with a stress stiffening mechanical response, PIC-based hydrogels form a unique platform to systematically investigate the influence of biophysical properties in cellular behavior.



PIC polymers display a β-sheet helix possessing a stiff backbone stabilized by hydrogen bindings between the side chains. They are acquired via a nickel(II) catalyzed polymerization of poly glycol functionalized isocyano-(D)-alanyl-(L)- alanines. Labeling the structure is done by targeting N3 functions using dyes linked to DBCO.

Structural characterisation

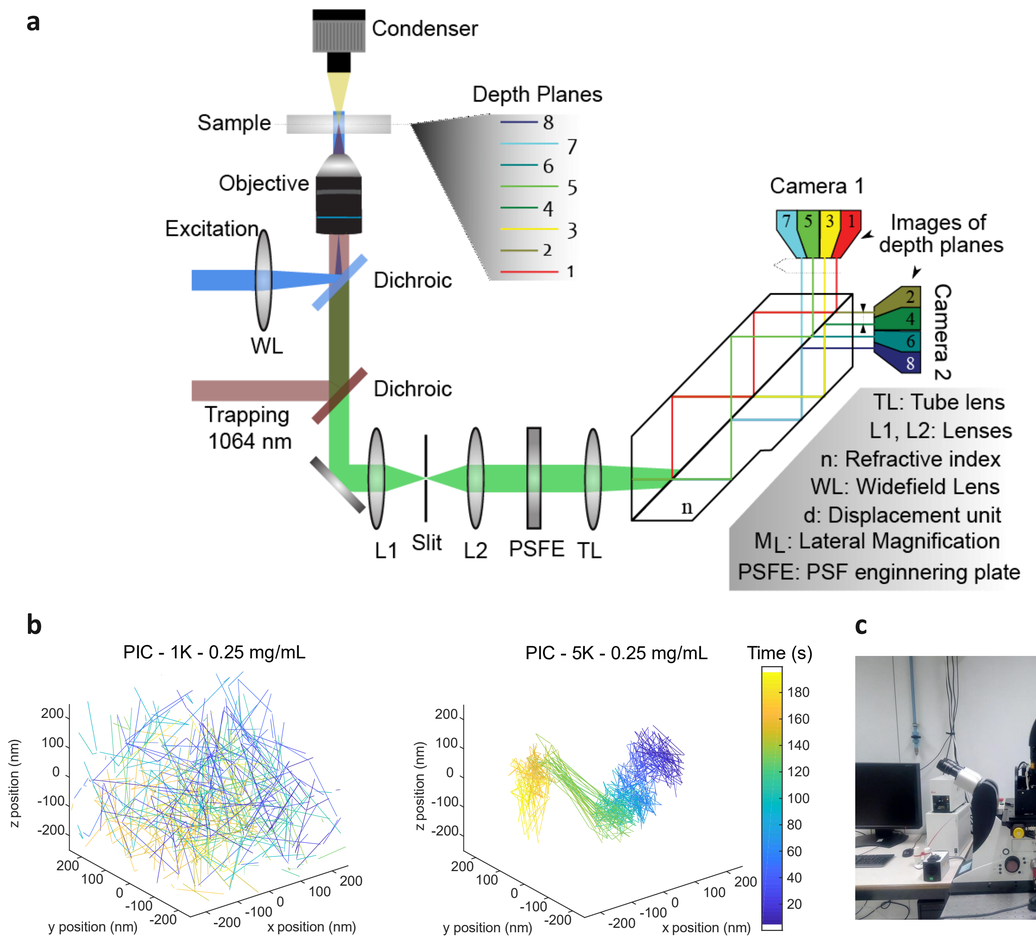
Fluorescence images were acquired for different PIC hydrogels and analyzed with an in-house program developed in MATLAB®. Thereafter several structural parameters can be calculated.



(a) Images were acquired using a confocal microscope. The structure display a heterogeneous, porous three dimensional structure. These images were then segmented based on intensity. **(b)** Out of the segmented images the pore diameter (the maximum diameter of a pore), throat diameter (the length of a connection or throat between pores) and pore connectivity (number of pores that are connected) was calculated. The throat diameter increases with a decreasing polymer concentration while the throat is rather independent of the concentration. When observing the connectivity there is also a decreasing trend when more polymer is present in the gel.

Mechanical characterisation

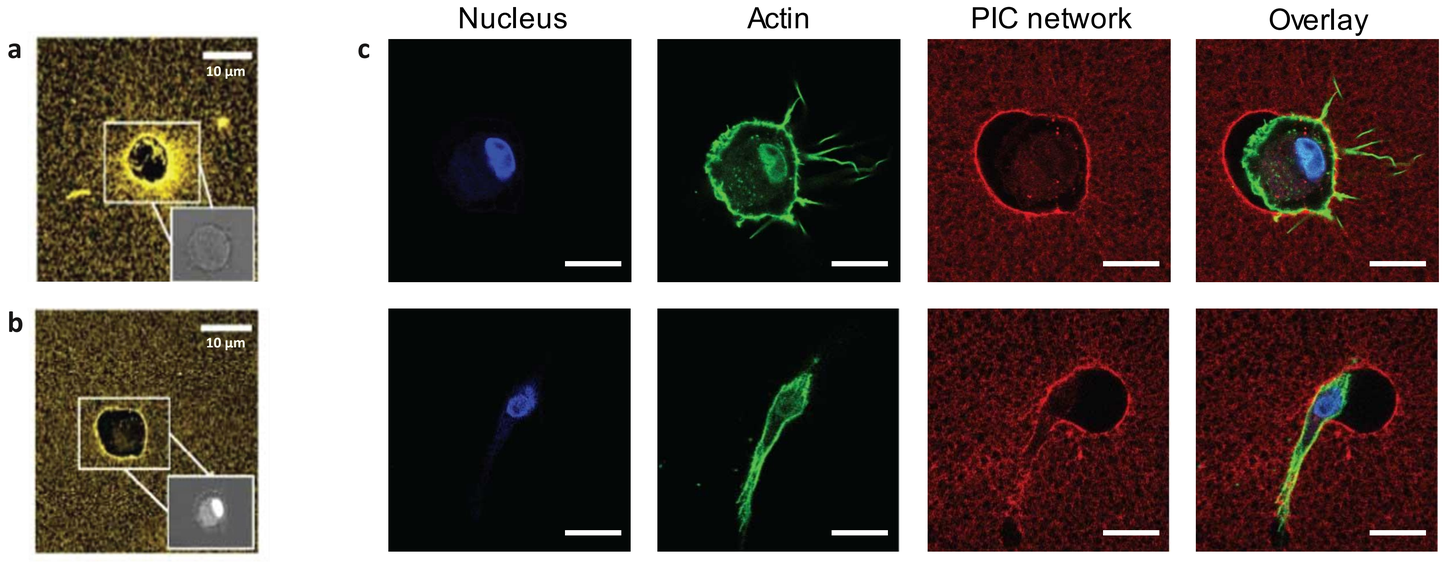
In addition to standard rheology, another way to investigate the mechanical properties of PIC hydrogels is to track the movement of beads trapped in the gel. Therefore an inhouse multiplane widefield setup is developed, allowing us to track particles in different depth planes at the same time. Movements of the beads will be influenced by its surrounding environment in such a way it can give crucial information of local rheological properties of the gel.



(a) A schematic representation of the custom build multi plane widefield setup, used to track beads in 3D. **(b)** Different polymer lengths result in specific behavior of beads due to a difference in stiffness. More uniform traces are observed in stiffer hydrogel. **(c)** The data acquired from the multiplane widefield setup can be compared with micro rheology data measured on a rheo-confocal setup.

Cell-mediated matrix remodelling

Visualization of the polymer fibers in the gel allows us to investigate fiber remodeling induced by stem cells. Here, PIC molecules were rendered biocompatible by adding RGD-peptides in the side chain. These peptides can be recognized by the cells, allowing for their proliferation and migration. In order to create space to facilitate migration, cells remodel the surrounding PIC network. The amount of proliferation was measured using a colorimetric assay (MTT assay).



(a) When PIC is enriched with RGD-peptides cells can interact with their surrounding matrix. **(b)** Without the presence of RGD-peptides cells are not able to spread. **(c)** Human derived adipose stem cells encapsulated in PIC-RGD hydrogel (1 mg/ml, 5K) were fixed and stained after one day (*top row*) and 7 days (*bottom row*). Scalebar 20 µm. **(d)** HeLa cells were encapsulated in PIC hydrogel (5K) without RGD-peptides. After 7 days an MTT-assay was conducted to measure the proliferation rate. The graph displays an average of three experiments.

Conclusion

Introducing advanced microscopy methods into polymer research opens the door to unravel unknown mechanical and biological properties on a micro and nanoscale. The PIC hydrogel network under study displays a very heterogeneous porous structure. We observed a decrease in pore size with increasing polymer concentration. When PIC hydrogel is functionalized with RGD we observed proliferation and migration of stem cells through the hydrogel, accompanied by a modification of the polymer network.

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References & Acknowledgements: [1] P. Kouwer *et al.*, Nature, 2013. (doi: 10.1038/nature11839). This project has received funding from the FWO (project numbers: G0A5817N, 12J2616N and 1529418N).