Supporting Information to

3D Hydrogels Containing Interconnected Microchannels of Subcellular Size for Capturing Human Pathogenic *Acanthamoeba Castellanii*

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Elasticity of bulk samples: Bulk samples without ZnO were prepared according to the prescription above. The polymer mixture was poured in PTFE molds with a diameter of 10 mm and 5 mm height. The samples were polymerized at room temperature for 1 h and then treated with either bidest. water or 0.5 M hydrochloric acid (HCl) for 2 d. Afterwards, the samples were washed with bidest. water until the pH of the solution was neutral (up to 6 d). The swollen samples were measured with an indentation experiment (as described in more detail in the following paragraph). The results of the analysis of the Young's modulus is

presented in table S1 and show that HCl treatment does not have a significant impact on PAAm elasticity.

Table S1. Young's modulus of PAAm bulk samples after being incubated in bidest. water or 0.5 M HCl for 2 d and subsequently washed and swollen to equilibrium in bidest. water. The results show that the acid treatment does not significantly change the elasticity of the PAAm substrate.

Incubation Medium	Young's Modulus (kPa)
Bidest. water	18.6 ± 1.32
HCl (0.5 M)	18.9 ± 0.75

Detailed Description of Indentation Experiments to Determine PAAm Elasticity: The measurement setup is a modified tensile test setup. On one side a stepper motor (M229.26S, Physik Instrumente GmbH & Co. KG, Germany) introduces a movement and on the other side a load cell (KD24s 10N, ME-Meßsysteme GmbH, Germany) detects a force. To switch this setup to a tensile test setup a PTFE-sphere with a diameter of 6 mm was mounted to the moving arm of the stepper motor and a flat PTFE plate was mounted to the load cell. PTFE was chosen to reduce the friction between the surfaces and the sample.

For the measurement the sphere was moved to a predefined position above the sample. Afterwards it was lowered 3 mm with a velocity of 0.1 mm/s, resulting in an indentation of the sample. The indentation depth varied between the samples, because start- and endpoint of the measurement were predefined and the sample thickness varied.

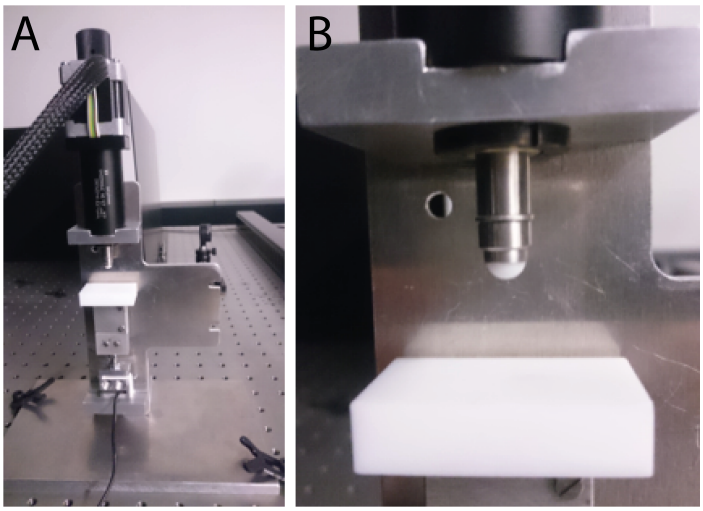

Figure S1. Photographs of the indenter setup used for the experiments. A) The complete setup is shown. On the bottom part a PTFE plate is mounted to a load cell to provide a surface for the indentation test and to measure the introduced force. On the top part a PTFE sphere is mounted to a stepper motor to introduce an indentation. B) Magnified view of sphere and surface. The sample is placed on the surface and penetrated by the sphere.

Force and indentation depth data were processed in Matlab R2015a. The mean force of the first 10% of the data points (far away from the sample) was subtracted from the force-data as a baseline. The lsqcurvefit function from the Matlab Curve Fitting Toolbox Version 3.5.1 was used to fit the Hertz Model to the data. The stiffness of the indenter is assumed to be much higher than that of the sample. By limiting the fitting range successively, we computed how the measured Young's modulus (E) changes with depth of indentation. We see a rapid increase of Young's modulus followed by a saturation which shows as a plateau in the E vs distance curve (see Fig. S3). Only E values in this region were assumed to present the samples properties properly. Hence the Hertz model was used for fitting the data. It is expressed as:

$$F = \frac{4E\sqrt{R}}{3(1-\upsilon^2)}(\delta - \delta_0)^{\frac{3}{2}}$$

With F as Force, E as Young's modulus and υ as Poisson's ratio of the hydrogel, R as indenter radius, δ as indentation depth and δ_0 as contact point. E and δ_0 are the fitting parameters. A Poisson's ratio of 0.5 is assumed for the hydrogels.[1]

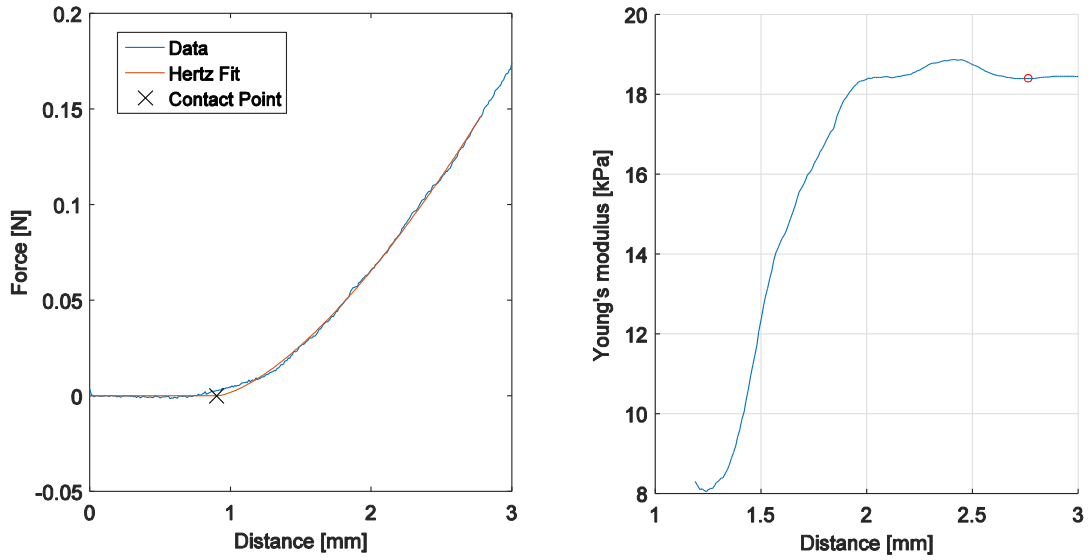

Figure S2. Left: A typical Force-distance curve of an indentation experiment. The blue line shows the measured data and the red line presents the Hertz model fit. The black X is the contact point, which is one of the fitting parameters. Right: By limiting the fitting range successively, we computed a Young's modulus-distance curve. It clearly shows an increase of Young's modulus with indentation depth. After an indentation of approximately 2 mm, a saturation can be observed and Young's modulus remains constant upon further indentation. The red circle indicates the value we chose to represent the elastic properties of the sample.

Gel content and swelling of bulk samples: For determination of the gel content and the degree of swelling bulk samples were lyophilized and weighted (W_0). The samples were treated

[1] Schwarz, U. S.; Balaban, N. Q.; Riveline, D.; Bershadsky, A.; Geiger, B.; Safran, S. A., Calculation of forces at focal adhesions from elastic substrate data: the effect of localized force and the need for regularization. Biophysical Journal 2002, 83 (3), 1380-1394.

either with 3 mL bidest. water or 0.5 M HCl acid for 2 d. Afterwards, the hydrogels were washed with bidest. water until the pH of the solution was neutral and weighted (W_S). The samples were lyophilized and weighted again (W_1). The gel content was calculated according to the following equation:

$$Gel\ content\ (\%) = 100 \cdot \frac{W_1}{W_2}$$

The degree of swelling was calculated by the following equation:

$$Degree\ of\ swelling\ (\%) = 100 \cdot \frac{W_s - W_1}{W_1}$$

The results are summarized in table S2 and show that the HCl treatment does not have a significant impact on the swelling behavior or the gel content of PAAm.

Table S2. Gel content and degree of swelling of PAAm bulk samples after being incubated in bidest. water or 0.5 M HCl for 2 d and subsequently washed and swollen to equilibrium in aqua bidest. The results show that the acid treatment does neither change the gel content nor the swelling behavior of the PAAm substrate significantly.

Incubation Medium	Gel Content (%)	Degree of Swelling (%)
Aqua Bidest.	98.2 ± 1.04	2018 ± 25
HCl (0.5 M)	98.1 ± 0.54	2048 ± 24

Supplementary Movie 1: 16 h time-lapse of *Acanthamoeba castellanii* moving through 3D hydrogel channel network with embedded cAMP solution.

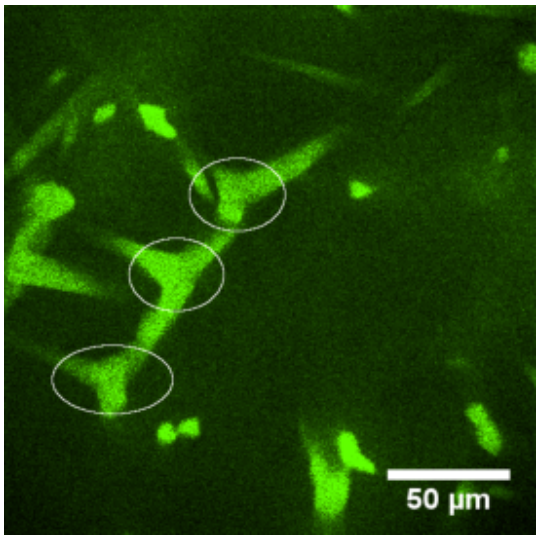

Figure S3. Fluorescence microscopy image of a microchannel-containing PAAm. The channels were filled with FITC dextran in order to render them fluorescent. The white circles indicate junctions in the microchannel network.

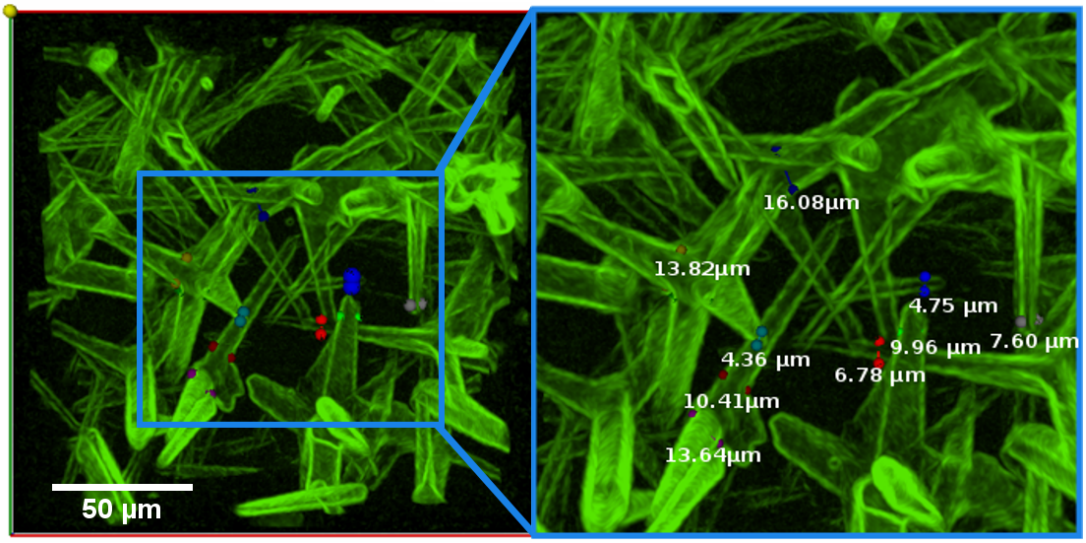

Figure S4. Top-view of a composition of z-stack images of a PAAm sample containing microchannels, which are filled with FITC dextran. Using z-stacks of fluorescent microscopy images of fluorescent microchannels, the channel diameters can be determined using image processing softwares. The zoom on the right shows exemplary results of measured channel diameters.