## Electronic supplementary information for the manuscript

# Stress relaxation amplitude of hydrogels determines migration, proliferation, and morphology of cells in 3-D

<sup>a</sup>.Institute of Biomaterials, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany.

<sup>b.</sup> Department of Physics, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany.

<sup>c</sup> Institute for Polymer Materials, University of Erlangen-Nürnberg, Martensstraße 7, 91058, Erlangen, Germany. \*Correspondence: ben.fabry@fau.de

Jonas Hazur, <sup>a</sup> Nadine Endrizzi, <sup>b</sup> Dirk W. Schubert, <sup>c</sup> Aldo R. Boccaccini<sup>a</sup> and Ben Fabry\*<sup>b</sup>



Figure ESI 1: Correlation between Young's moduli and motile fraction of cells, migration speed, circularity, and cell proliferation on day 7 (left) and day 10 (right). Rank correlation r and its significance value p are indicated in the graphs. Black lines are a fit of Eq. 2 to the data as a "guide to the eye" (shown only for statistically significant correlations).

#### Correlation of Young's modulus and cell behavior



### Correlation of $\tau_{0.75}$ or $\tau_1$ and cell behavior

Figure ESI 2: a) Time to relax the stress to 75 % of its initial value ( $\tau_{a,75}$ ) and b) its correlation to motile fraction of cells, migration speed, circularity, and cell proliferation on day 7 (left) and day 10 (right). c) Correlation between relaxation time  $\tau_1$  (generalized Maxwell model) and motile fraction of cells, migration speed, circularity, and cell proliferation on day 7 (left) and day 10 (right). c) Correlation between relaxation time  $\tau_1$  (generalized Maxwell model) and motile fraction of cells, migration speed, circularity, and cell proliferation on day 7 (left) and day 10 (right). Rank correlation r and its significance value p are indicated in the graphs. Black lines are a fit of Eq. 2 to the data as a "guide to the eye" (shown only for statistically significant correlations).

### **Creep recovery experiments**



Figure ESI3: a) Creep-relaxation behaviour of non-, medium- and high-pre-crosslinked ADA-GEL, Alg-ADA-GEL and Alg-GEL samples after 1 day of incubation. b) Hydrogels were loaded for 660 s with a constant stress of 6 Pa (loading phase), which subsequently was reduced to 0.4 Pa (unloading phase) for another 660 s. c) Plasticity was computed as the strain measured at the end of the unloading phase, normalized to the maximum strain at the end of the loading phase.

#### Representative cell images and videos

Time laps videos (12.5 h duration) of maximum intensity projections of NIH/3T3 tdTomato cells embedded in non-, medium- and high-pre-crosslinked ADA-GEL, Alg-ADA-GEL and Alg-GEL hydrogel samples after 1, 3, 7 and 10 days of culture. Videos can be downloaded via the following hyperlinks for ADA-GEL, Alg-ADA-GEL and Alg-GEL and Alg-GEL. Scale bar =  $50 \mu m$ .

	Day 1	Day 3	Day 7	Day 10
ADA-GEL			Jaco	
hign	17	· · · · ·		
medium	* • • • •			
no		• • •		
Alg- ADA-GEL high				
medium			••••	• • • •
no	0	•	•	
Alg-GEL	1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1. 1		2 8 4 C 6 -	
high				
medium				•
no	•		• •	• •

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Figure ESI 4: Maximum intensity projection images of NIH/3T3 tdTomato cells embedded in non-, medium- and high-precrosslinked ADA-GEL, Alg-ADA-GEL and Alg-GEL hydrogel samples after 1, 3, 7 and 10 to visualize proliferation. Scale bar = 50 \_\_μm.



Figure ESI 5: Maximum projection images of NIH/3T3 tdTomato cells embedded in non-, medium- and high-pre-crosslinked ADA-GEL, Alg-ADA-GEL and Alg-GEL hydrogel samples are shown for day 1, 3, 7 and 10 to visualize cell morphology. Red numbers depict mean roundness values of exemplary cells per image. Scale bar = 50 µm.