Physical Entanglement Hydrogels: Ultrahigh Water Content but Good Toughness and Stretchability

Fatih Puza, Yijun Zheng, Lu Han, Lulu Xue, Jiaxi Cui**

Supporting Information

Materials and Methods

Materials

Acrylamide (⩾99%) (AA), ammonium persulfate (APS), N,N'-methylenebis(acrylamide) (MBA), dioctyl sulfosuccinate sodium salt (AOT), n-Hexane, and tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich and used as received.

Fabrication of nanogels

In a typical example, AOT surfactant (1.59 g) was dissolved in n-Hexane (42 ml) under stirring and the mixture was purged by N_2 for 10 min. To this mixture, the solution of acrylamide (0.527 g) and MBA (0.15 g) in water (1.7 ml) was added under stirring to generate an emulsion solution. The emulsion was stirred at room temperature (25 °C) for 30 minutes and then to which, APS aqueous solution (30 µl with 100 mg/ml concentratio) and TEMED (15µl) were added to initiate polymerization at room temperature. The polymerization process was conducted under a stirring condition for 2h (IKA RCT basic, 900 rpm), followed by removing the n-hexane via evaporation in air. The residual solution was washed with ethanol and centrifuged at 4000 rpm for 5min. This process was repeated for 3 times to remove surfactant and unreacted residues. The obtained white material was subsequently dried at room temperature before further use.

Fabrication of PEH

The hydrogels were synthesized via free-radical polymerization. Take the synthesis of system PEH30-0.75 as an example. Briefly, nanogels (0.0023g, 0.75 wt% of monomer) were dispersed in distilled water (1 ml) under ultrasonication and purged by N_2 flow for 5min. To this solution, APS (0.009g, 3 wt% of monomer) were added and the mixture was stirred for 30min at room temperature (25 °C), followed by adding acrylamide monomer (0.3g) under stirring. The mixture was stored for overnight in vial at room temperature for hydrogelation. The detailed compositions of all used sample groups can be found in Table S1, Supporting Information. Therefore, acrylamide-only sample group was synthesized to evaluate importance of nanogels presence. For this aim, sample with 20 wt% monomer concentration was chosen. Briefly, 0.2g of acrylamide monomer was dissolved in distilled water, and followed by N_2 flow for 5min, prior to initiator supply. Then, APS (0.006g, 3 wt% of monomer) was added and solution was exposed to overnight reaction. Subsequently, gelation behavior of PEH20-0.75 (including nanogels), and acrylamide-only sample was compared.

The control AA-MBA hydrogel synthesized by following process. The mixture of acrylamide monomer (0.3g), MBA (0.0001g) in distilled (1ml) water was purged by N_2 flow for 5min, followed by adding APS (0.009g). The solution was cast on a mold (2mm depth) for hydrogelation. The top-side of the solution was covered with a glass slide to prevent oxygen infusion, and gelation was completed at room temperature.

Characterization Techniques

The chemical structure of the nanogels was investigated with ^{1}H NMR, performed on Bruker AC 300. The mean size and distribution of the nanogels were characterized by using dynamic

light scattering analysis device (Malvern Zetasizer Nano) at room temperature (25 °C). Briefly, the dried nanogels were dispersed into distilled water under ultrasonication for 5min and the analysis was conducted, immediately. Morphology of dried nanogels was studied with a transmission electron microscope (TEM, JEM 2100) at 200 kV. First, the dried nanogels were dispersed in ethanol under ultrasonication (5min), and applied onto a copper TEM grid. The diluted nanogel solution was dried at room temperature, prior to the imaging process.

Water absorption capability of hydrogels was evaluated by the weight change. The synthesized and freeze-dried hydrogels were immersed in distilled water at room temperature (25 °C). The swelling ratio was defined as:

$$Swelling\ Ratio(\%) = \frac{Wswollen - Wdry}{Wdry} \times 100$$

$$Polymer\ fraction\ (\%) = \frac{Initial\ Monomer\ Amount(\%)}{W24h\ swollen(\%)} \times 100$$

where the polymer fraction of hydrogels in the swollen state was defined as the ratio of initial monomer amount to total swollen hydrogel weight.

Scanning electron microscope (SEM-Quanta FEG 400) was employed to observe cross-sectional morphology of hydrogels in various states. The prepared/swollen hydrogels were first immersed in liquid nitrogen and then freeze-dried at −43 °C under a vacuum of 0.1 Pa for 24h. Prior to imaging, gold sputtering of freeze-dried samples was performed. The images were obtained with an accelerating power of 30kV.

Dynamic rheology and compression test measurements were performed on a HR-3 Rheometer equipped with a Peltier plate to control the temperature (TA Instruments). The temperature value was set to 25 °C before tests. The swollen hydrogel samples were cut with a 12mm-diameter puncher. For rheological measurements, the upper parallel plate with a diameter of

12mm was used and the distance of gap was set as 2 mm. The frequency sweep was performed between 1-10 Hz with 0.1% strain value. Strain amplitude sweeps were conducted in the range of 0.01-1000%, at 1 Hz. For the compression tests, the upper parallel plate with a diameter of 20mm was used and the gap was set as 2.5 mm before starting the compression. 10N loading force was used during the test and the samples were compressed for 2 minutes. The compression modulus was calculated by the values in the range of 10-40 % strains.

Molar mass distributions of polymer chains were measured by a gel permeation chromatography (GPC, Agilent HPC1100 Serie with RI-Detector), using a PSS-SUPREMA LUX Column with standard polystyrene as the reference. Prior to measurement, PEH hydrogel was exposed to continuous swelling and fully dissolved in water. Subsequently, the extensive water was removed out from dissolved product, and concentrated solution (4mg/ml) was obtained by dissolving polymers in mobile phase. Prior to measurement, the obtained solution was filtered by using a filter with pore size of 1μm. Polymer solutions with 20μl injection volume were supplied to the system with 1ml/min flow rate. Mobile phase of 0.1M NaCl and 0.3% TFA was used for the measurements. Therefore, molecular weights of nanogels were characterized by performing same GPC protocol.

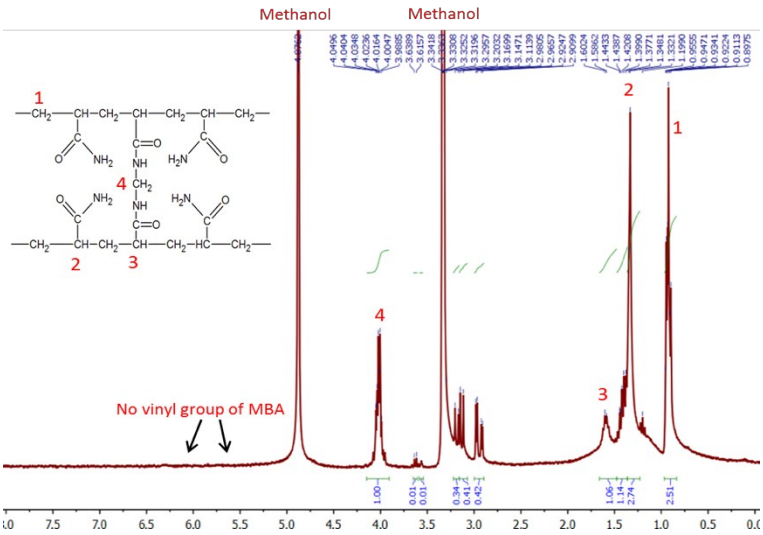

Fig. S1. ^{1}H NMR spectra of synthesized nanogels after complete purification steps.

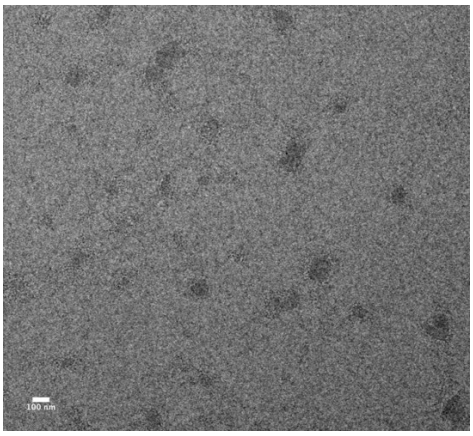

Fig. S2. Transmission electron microscope(TEM) image of synthesized polyacrylamide nanogels. Scale bar is 100nm.

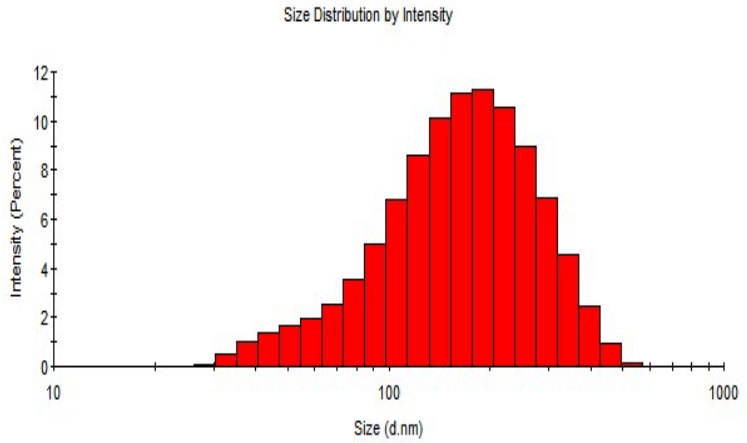

Fig. S3. Size distribution of synthesized nanogels in swollen state, characterized via dynamic light scattering.

Table S1. Chemical compositions of synthesized hydrogels.

Sample Code	Monomer (Acrylamide)		Nanogel		Initiator (APS)		Water
	Ratio/Amount		**Ratio/Amount**		**Ratio/Amount**		
PEH20-0.75	20 wt%	0.2g	0.75 wt%	0.0015g	3 wt%	0.009g	1ml
PEH30-0.37	30 wt%	0.3g	0.37 wt%	0.0011g	3 wt%	0.009g	1ml
PEH30-0.75	30 wt%	0.3g	0.75 wt%	0.0023g	3 wt%	0.009g	1ml
PEH30-1.5	30 wt%	0.3g	1.5 wt%	0.0046g	3 wt%	0.009g	1ml
PEH40-0.75	40 wt%	0.4g	0.75 wt%	0.003g	3 wt%	0.012g	1ml
AA-MBA	30 wt%	0.3g	0.03 wt% MBA	0.0001g MBA	3 wt%	0.009g	1ml

* The coding of the samples was done considering monomer and nanogel ratio, respectively.

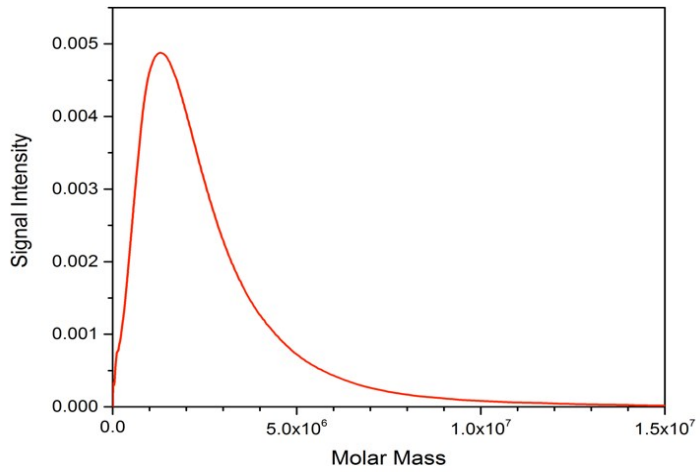

Fig S4. Molar mass distribution of fully dissolved hydrogel, obtained from GPC.

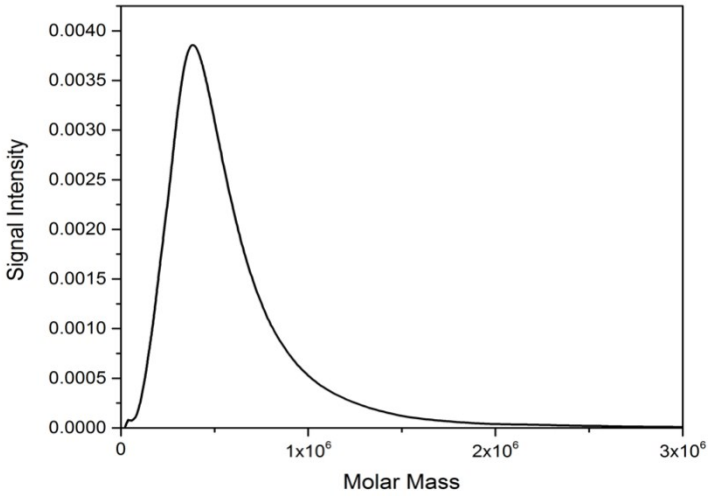

Fig S5. Molar mass distribution of nanogels, obtained from GPC

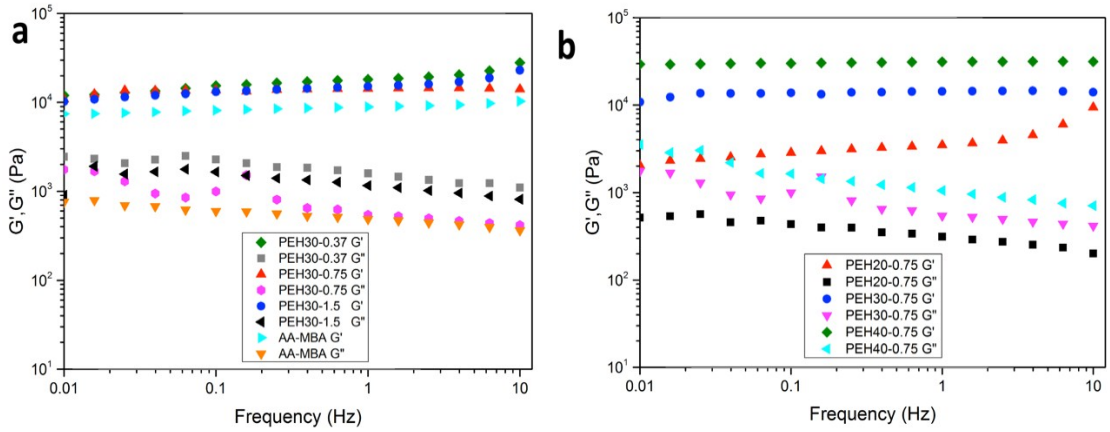

Fig. S6. Frequency sweep of as-prepared PEHs and AA-MBA hydrogel. (a) Nanogel concentration and covalent cross-linking influence on rheological properties (b) Change of G' and G'' by altering monomer concentration in the feeding solution.

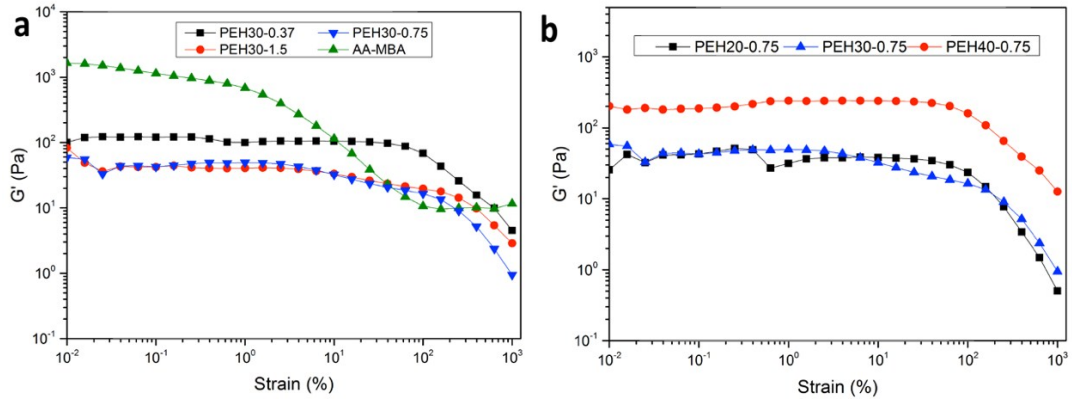

Fig. S7. Strain sweep measurement of all synthesized hydrogels. Polymer fractions of PEHs were set to 2%. (a) Nanogel concentration and covalent cross-linking dependency of strain-thinning nature. Dynamic PEHs are able to suppress deformation at higher strain levels. (b) Strain-thinning behavior of PEHs can be tuned by increasing feeding monomer concentration.

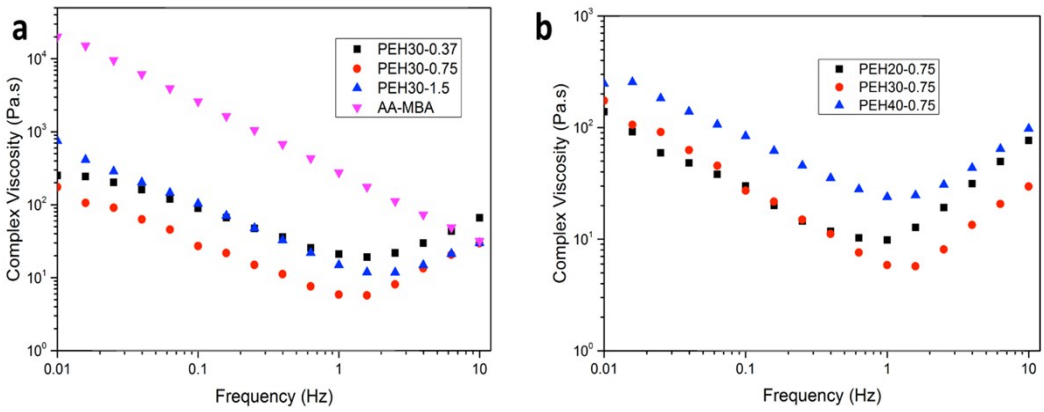

Fig. S8. Complex viscosity vs. Frequency sweep of as-prepared PEHs and AA-MBA hydrogel. Polymer fractions of PEHs are 2%, while AA-MBA is only able to reach 8% (a) Nanogel concentration and covalent cross-linking dependency of complex viscosity (b) Influence of monomer concentration on complex viscosity change.

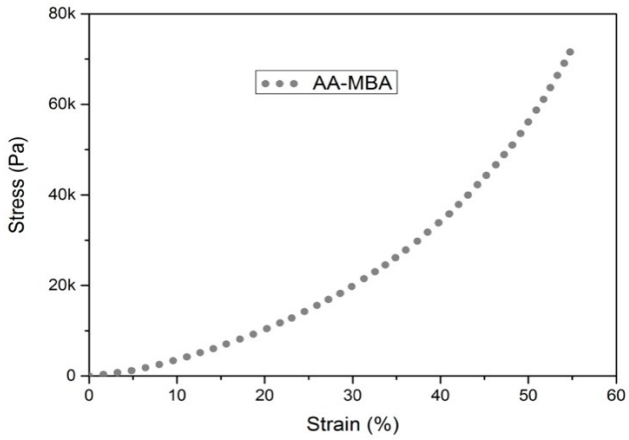

Fig. S9. Compression test of swollen AA-MBA with 8wt% polymer fraction.

It is challenging to improve the stretching ability for a high-water-content hydrogel. In swollen state, PEH show high stretching ability, compared to AA-MBA control. Moreover, same phenomenon was observed in case of as-prepared PEH and AA-MBA samples. The stretchability of PEH30-0.75 and AA-MBA after gelation was demonstrated in Fig. S10. According to results, PEH30-0.75 was able to be stretched two times higher than covalently bonded hydrogel. This results are attributed to unique nature of physical entanglement crosslinking. The physical entanglements were able to slide inside movable nanogels and

dissipate energy in a better way during stretching process.

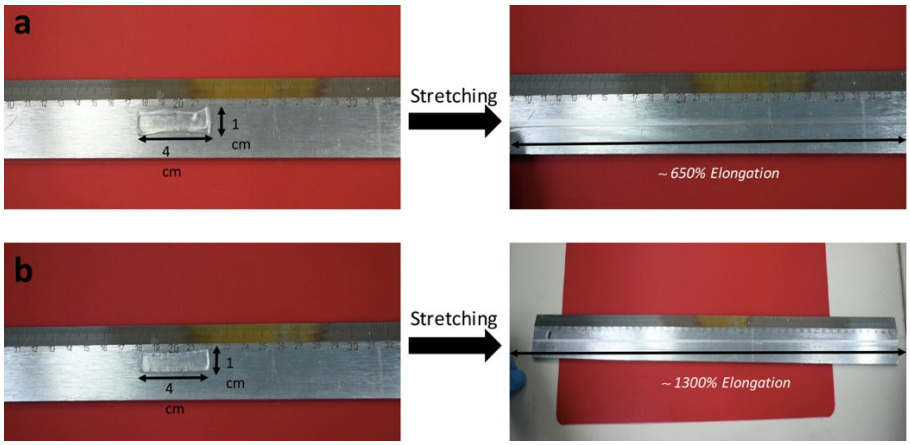

Fig. S10. Demonstration of PEH30-0.75 and AA-MBA samples in as-prepared state, before water intake. (a) Covalently cross-linked AA-MBA was able to be stretched ~6.5 times of initial length. (b) The elongation capability of PEH30-0.75 was greatly higher with ~13 times stretching.

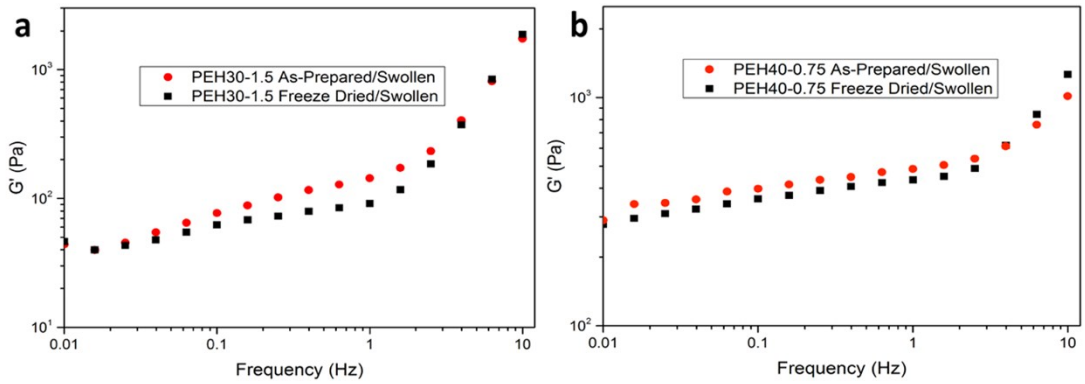

Fig. S11. Frequency sweep (within 0.01-1 Hz) of extra PEHs, which also can recover G' after rehydration of freeze dried state. As-prepared and freeze dried specimens were swollen in water for 24h and rheological properties were investigated. (a) Frequency dependent G' of PEH30-1.5 (b) PEH40-0.75 with higher monomer ratio.

In vitro Cell Study

To demonstrate possible application for PEH system, in vitro cell study was performed. The dynamic network of PEH is supposed to allow cell penetration inside hydrogel. With considering that, we synthesized a hydrogel by using same PEH mechanism. Basically, hydrogel was synthesized with composition of 300 µl N,N-Dimethylacetamide (DMA) and 100µl acrylic acid as monomers. 1.5wt% acrylamide nanogel was supplied as cross-linker and polymerization was completed in 1ml water with the help of 3wt% APS. The polymerization was completed at 35 °C. Prepared hydrogels were overnight swollen in cell medium to remove unreacted residues and then RGD surface functionalization was conducted.

RGD surface functionalization

The hydrogels were incubated in an aqueous solution of 0.2 M EDC (N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride), 0.1 M NHS (N-hydroxysuccinimide), 2-(N-morpho)-ethanesulfonic acid (0.1 M) and NaCl (0.5 M). After 15 min, the solution was removed and the hydrogel were washed with deionized water and incubated in a solution of cyclo(RGDfK) (0.5 mg/mL) for 1h. The substrates were afterwards rinsed with PBS and used immediately for cell experiments.

Cell culture

Jurkat cell, Clone E6-1 (ATCC® TIB-152™) were cultured in ATCC-formulated RPMI-1640 Medium supplemented with 10% fetal bovine serum (Gibco), 100 units/mL penicillin, and 100 mg/mL streptomyocin at 37°C, 5% CO_2. Medium were renewed every 48 h.

Migration Assay

For the transwell migration experiments, RGD modified hydrogels with a thickness of 3 mm were cut and plated on the PET membrane of 12 mm transwell inserts (greiner bio-one, NY, cat. no. 665630, pore size: 3 µm,). The hydrogel was sterilized with ethanol and rinsed 3 times with PBS. The wells of the receiver plate were filled with RPMI medium supplemented with 10% (v/v) FCS and 50 ng mL^{-1} CXCL12 (Thermo Fisher Scientific) before the transwell inserts containing the hydrogels were inserted into the receiver plate. Subsequently, Jukart T

Cells in RPMI medium supplemented with 2% (v/v) FCS were seeded on top of the hydrogels. The following volumes and cell numbers were used: 12 mm transwells: receiver well: 1 mL, insert: 500 µL, cells per transwell: 7 x 10^5 each (living and dead).

Life and Dead Assay

Cell culture medium was removed and samples were incubated for 5 min with fluorescein diacetate (40 µgmL^{-1}, Sigma, F7378) and propidium iodide (30 µgmL^{-1}, Sigma, P4170) in PBS. Samples were washed twice with PBS and imaged with Zeiss LSM 800 confocal microscope.

Results of Cell Study

Jurkat cells was selected as demonstration, since they are frequently used as model mammalian cells for the encapsulation studies in hydrogels. The cells were seeded onto hydrogel, and imaged after short term cell culture (two days). The confocal microscope imaging showed that cellular penetration inside 3D PEH hydrogel network is possible. The strategy of PEH allows to obtain dynamic hydrogel network with soft nature. With the help of such bulky nature, cells can regulate dynamic soft hydrogel network during their migration and create free spaces to migrate after 2 days. The abundant number of cells had ability to migrate inside of bulky matrix around 200 µm. Even though DMA-AAc based hydrogel did not show great biocompatibility, cells were still able to migrate inside. We believe that the sliding phenomena, which comes with PEH strategy and leads to soft matrix, is a promising tool to obtain 3D cell culture environments. By utilization of different polymer systems with PEH strategy can help to enhance biocompatibility, as well.

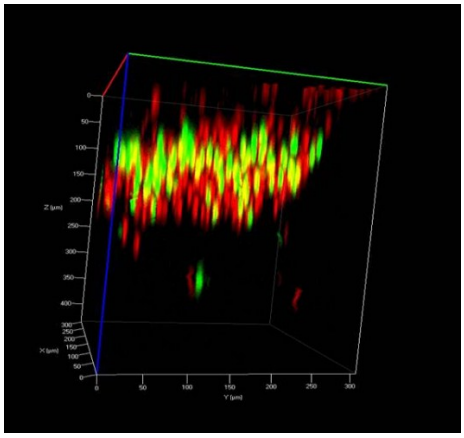

Fig. S12. Confocal Images of T Cells after 2 days seeding. The hydrogel network provides good conditions for cellular migration.