**SUPPLEMENTARY INFORMATION**

# BROADLY APPLICABLE HYDROGEL FABRICATION PROCEDURES GUIDED BY YAP/TAZ-ACTIVITY REVEAL STIFFNESS, ADHESIVENESS AND NUCLEAR PROJECTED AREA AS CHECKPOINTS FOR MECHANOSENSING

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## TABLE S1

| [1]AA wt% | [2]BA wt% | PAA Hydrogel | Elastic modulus PAA | PAA-OH Hydrogel | [3]Elastic modulus PAA-OH |
|---|---|---|---|---|---|
| 3.5 | 0.03 | PAA1 | - | PAA-OH1 | 0.32 ± 0.03 |
| 3 | 0.15 | PAA2 | 1.37 ± 0.22 | PAA-OH2 | 3.15 ± 0.27 |
| 5 | 0.15 | PAA3 | 4.47 ± 1.19 | PAA-OH3 | 6.09 ± 1.35 |
| 5 | 0.225 | PAA4 | 8.44 ± 0.82 | PAA-OH4 | 13.39 ± 1.73 |
| 8 | 0.48 | PAA5 | 40.40 ± 2.39 | PAA-OH5 | 50.01 ± 3.07 |

**Table S1:** Formulation and elastic moduli of PAA and PAA-OH hydrogels measured with micropipette aspiration (PAA-OH)[40] or from ref [11] (PAA).
[1] AA represent the sum of the monofunctional acrylates Acrylamide and N-hydroxyethyl acrylamide (HEA) for PAA-OH gels, and Acrylamide alone for PAA gels. The amount of HEA is fixed at 0.1M.
[2] BA wt% is the weight percent of bisacrylamide
[3] Stiffness measurements by micropipette aspiration.[40] The data indicate that the effect of the OH groups presence is to increase gel stiffnesses.

## TABLE S2

| | 10 gels | | | 30 gels | | | 50 gels | | |
|---|---|---|---|---|---|---|---|---|---|
| | **PAA** | **PAA-OH** | **PEG-RGD** | **PAA** | **PAA-OH** | **PEG-RGD** | **PAA** | **PAA-OH** | **PEG-RGD** |
| **Non-adhesive substrate functionalization (min)** | 30 | 0 | 145 | 60 | 0 | 180 | 90 | 0 | 220 |
| **Gel synthesis (min)** | 35 | 35 | 30* | 50 | 50 | 90* | 90 | 90 | 150* |
| **Protein functionalization (UV exposure and protein addition) (min)** | 30* | 2 | 0 | 90* | 5 | 0 | 150* | 10 | 0 |
| **Drying (min)** | 0 | 20** | 0 | 0 | 75** | 0 | 0 | 120** | 0 |
| **UV sterilization (min)** | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| **Total time (minutes)** | **110** | **72** | **190** | **215** | **145** | **285** | **345** | **235** | **385** |

**Table S2** Estimated time for the main steps that differs between PAA, PAA-OH and PEG-RGD gels for the synthesis and functionalization of 10, 30 or 50 hydrogels. For PAA, PAA-OH and PEG-RGD, times are calculated taking the most time-consuming formulations for each step. For the synthesis of PAA and PAA-OH, the non-adhesive substrate considered are functionalized glass and Kapton foil respectively. The PAA functionalization protocol is the covalent linking using sulfo-sanpah and considering a UV lamp with a homogeneous area of 100 $cm^2$. Timings are estimated according to published protocols.[11] For (*), multiple rounds with 5 hydrogels processed simultaneously are considered. For (**), multiple rounds with 10 hydrogels processed simultaneously are considered.

## TABLE S3

| **PEG-RGD** | | | | | |
|---|---|---|---|---|---|
| **Stiffness (kPa)** | 0.3 | | 2.6 | | 13.7 |
| **Mesh size (nm)** | 60-100 | | 23-32 | | <9 |
| **PAA-OH** | | | | | |
| **Stiffness (kPa)** | 0.32 | 3.15 | 6.09 | 13.39 | 50.01 |
| **Mesh size (nm)** | 60-100 | 23-32 | 9-12 | 9-12 | <9 |

**TABLE S3:** values of mesh sizes cut-off of PEG-RGD and PAA-OH hydrogels evaluated analyzing the diffusion of fluorescent dextrans or nanoparticles using a confocal microscope

## FIGURE S1

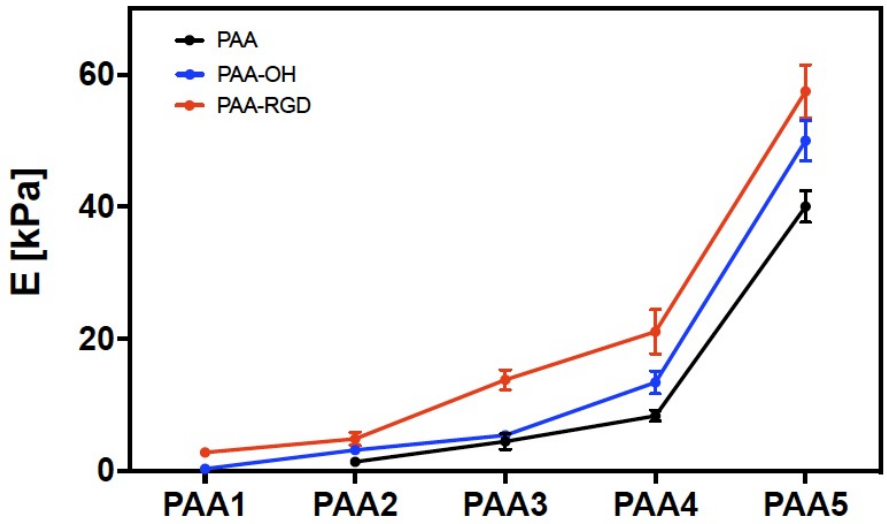


**Figure S1:** Elastic moduli of PAA and PAA-OH and PAA-RGD hydrogels measured with micropipette aspiration (PAA-OH, PAA-RGD)[40] or from ref [11] (PAA).

## FIGURE S2

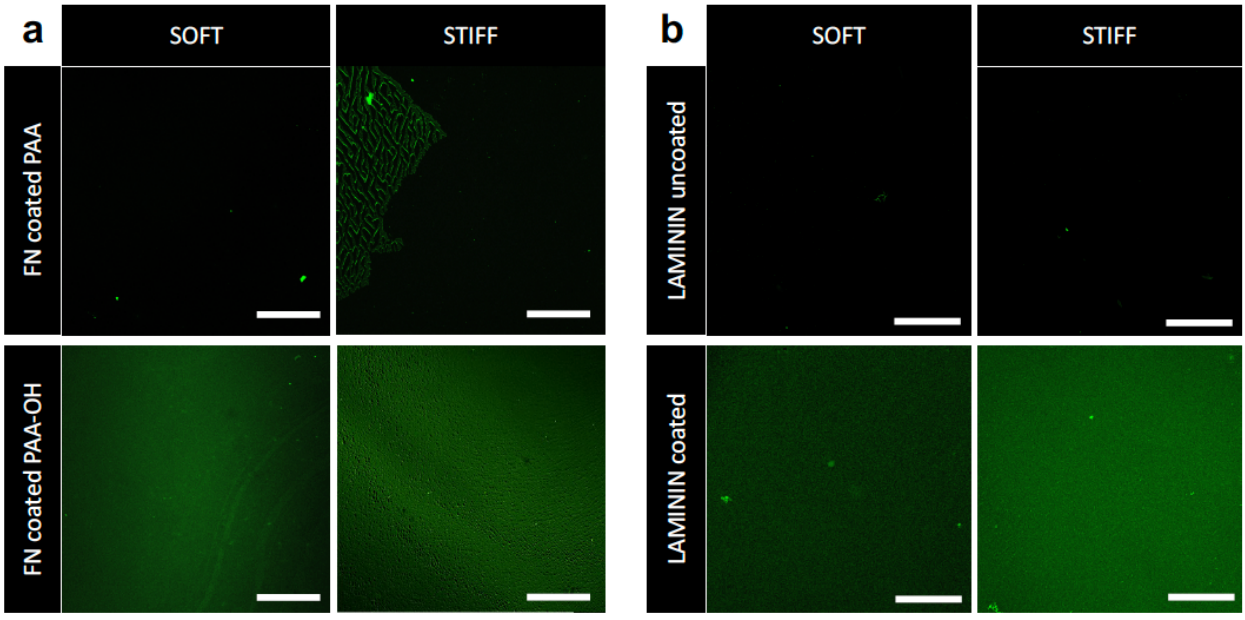


**Figure S2**: **a)** Fluorescence confocal images of FN and Alexa488 conjugated Fibrinogen coated on PAA (top) and PAA-OH (bottom) hydrogels soft (0.21kPa for PAA and 0.32kPa for PAA-OH) and stiff (40kPa for PAA and 50kPa for PAA-OH).
**b)** Fluorescence confocal images of PAA-OH hydrogels soft (left) and stiff (right) with and without laminin coating (25 µg/mL in PBS). Scale bar = 200 µm

**FIGURE S3**

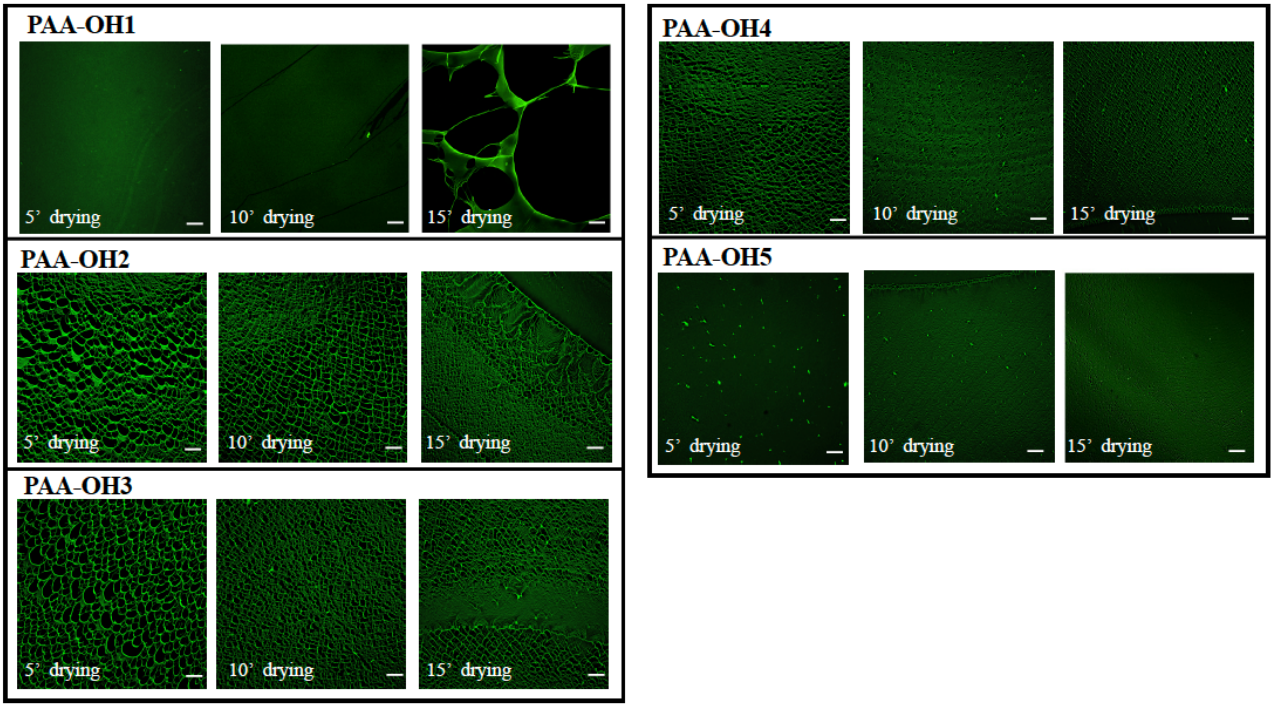


**Figure S2:** Fluorescence images of the PAA-OH substrates coated with a solution of fibronectin (25ug/mL) and Alexa488 conjugated Fibrinogen (2 µg/mL), for different stiffnesses and drying timing before cell seeding. Scale bar = 100 µm.

**FIGURE S4**

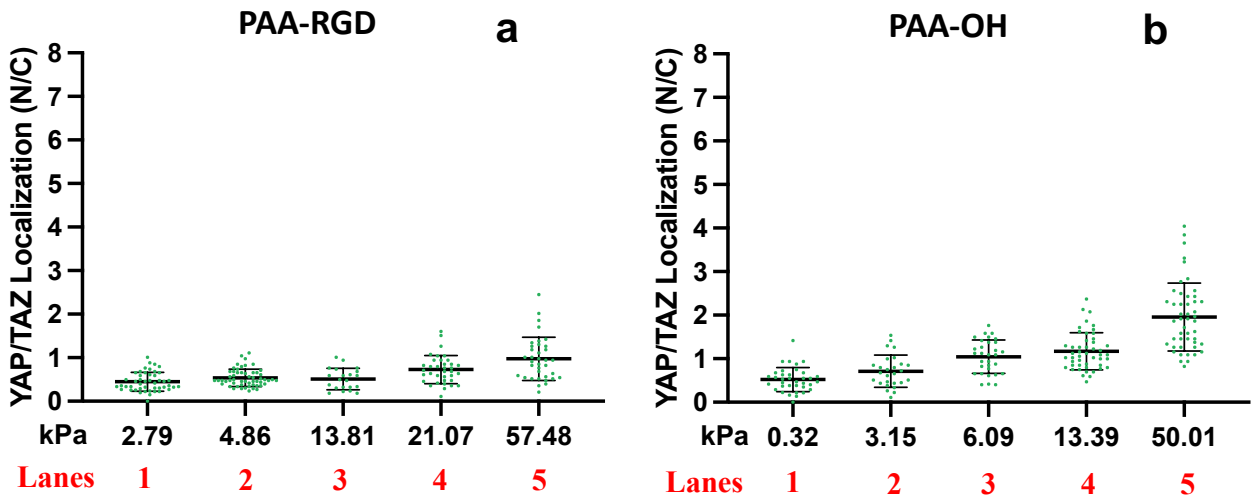


**Figure S4:** quantifications of the nuclear/cytoplasmic ratio of YAP/TAZ subcellular localization in U2OS cells, after seeding on PAA-RGD (a) or FN coated PAA-OH (b) substrates, of five different stiffness. YAP/TAZ levels are, in absolute values, reduced when PAA-OH and PAA-RGD substrates with the same stiffness are compared (compare lines 2, 3 and 5 of PAA-RGD with lines 2, 4 and 5 of PAA-OH). Number of cells for each lane is: a) lane 1:47; lane 2:55; lane 3:21; lane 4:36; lane 5:36 b) lane 1:36; lane 2:27; lane 3:31; lane 4:48; lane 5:48

**FIGURE S5**

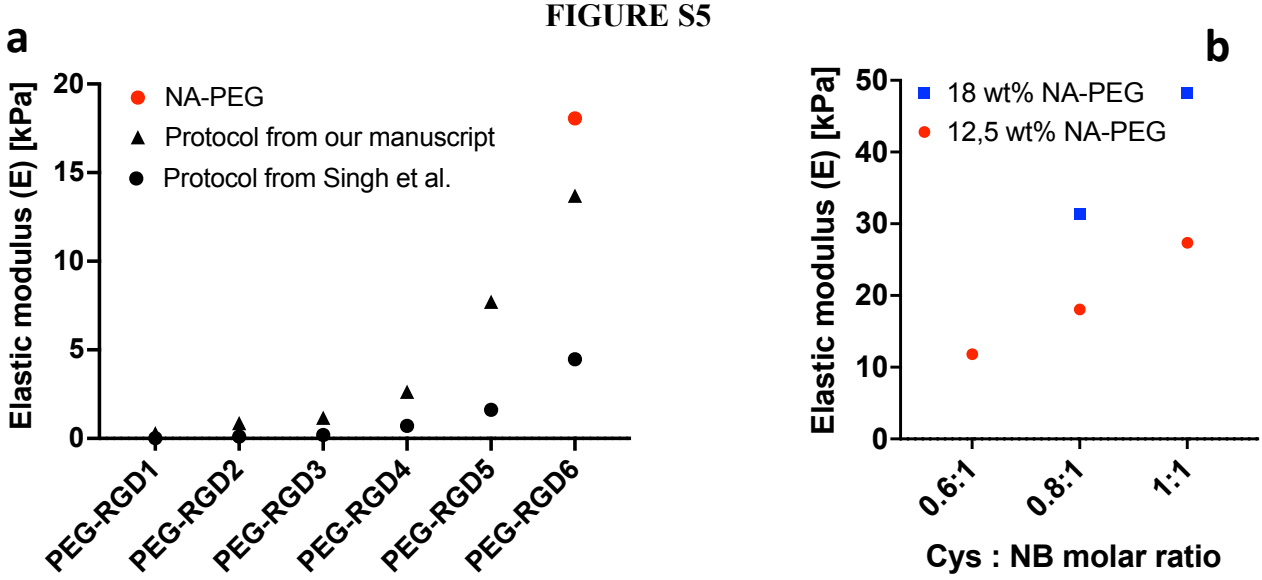


**Figure S5**: **a)** Mechanical properties (elastic modulus) measured with micropipette aspiration of PEG-RGD formulations prepared with the synthesis reported in literature[35] (prereaction step between PEG and RGD containing peptides) and the synthesis adopted in our work. In red, it is reported also the stiffness of a non-adhesive gel with the same composition of PEG-RGD6 but prepared without the presence of RGD peptide (called NA-PEG). **b)** Mechanical properties (elastic modulus) measured with micropipette aspiration of PEG non-adhesive gels (NA-PEG) prepared without the presence of RGD peptide and different concentrations of PEG and Cys:NB molar ratios.

## FIGURE S6

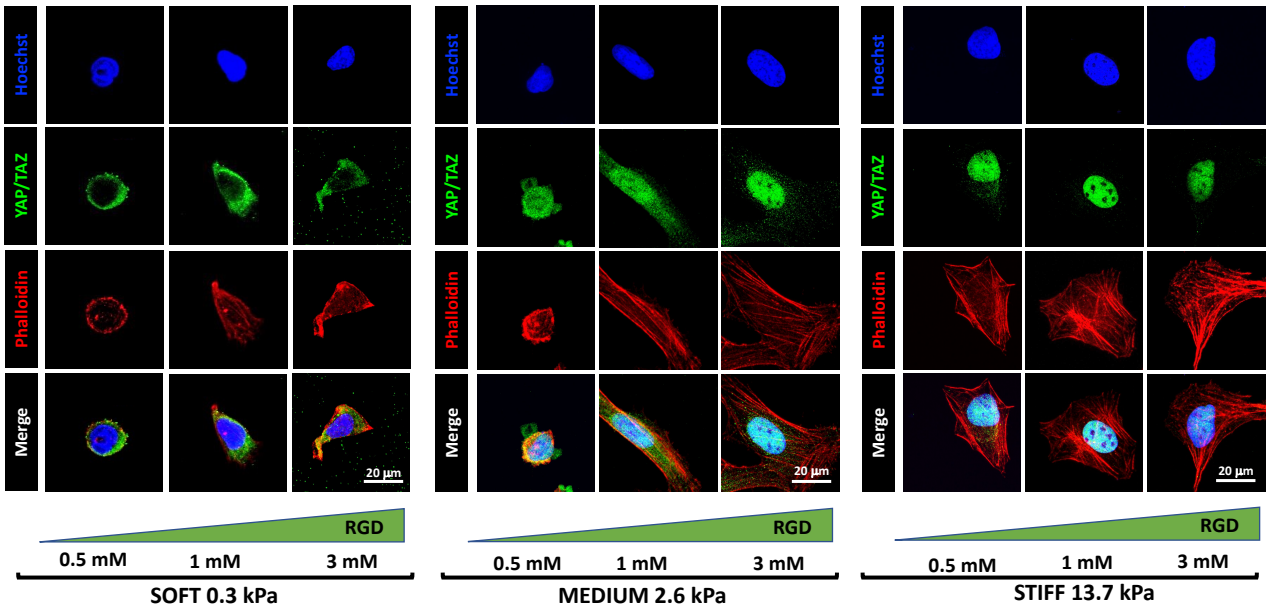


**Figure S6:** Immunofluorescence of U2OS (one representative cell) plated on PEG-RGD hydrogels at three different values of stiffness and RGD concentration. From the staining are visible: nuclei (in blue), F-actin (in red), YAP/TAZ (in green). F-actin was stained with fluorescently-labeled phalloidin to serve as cell shape reference.

## FIGURE S7

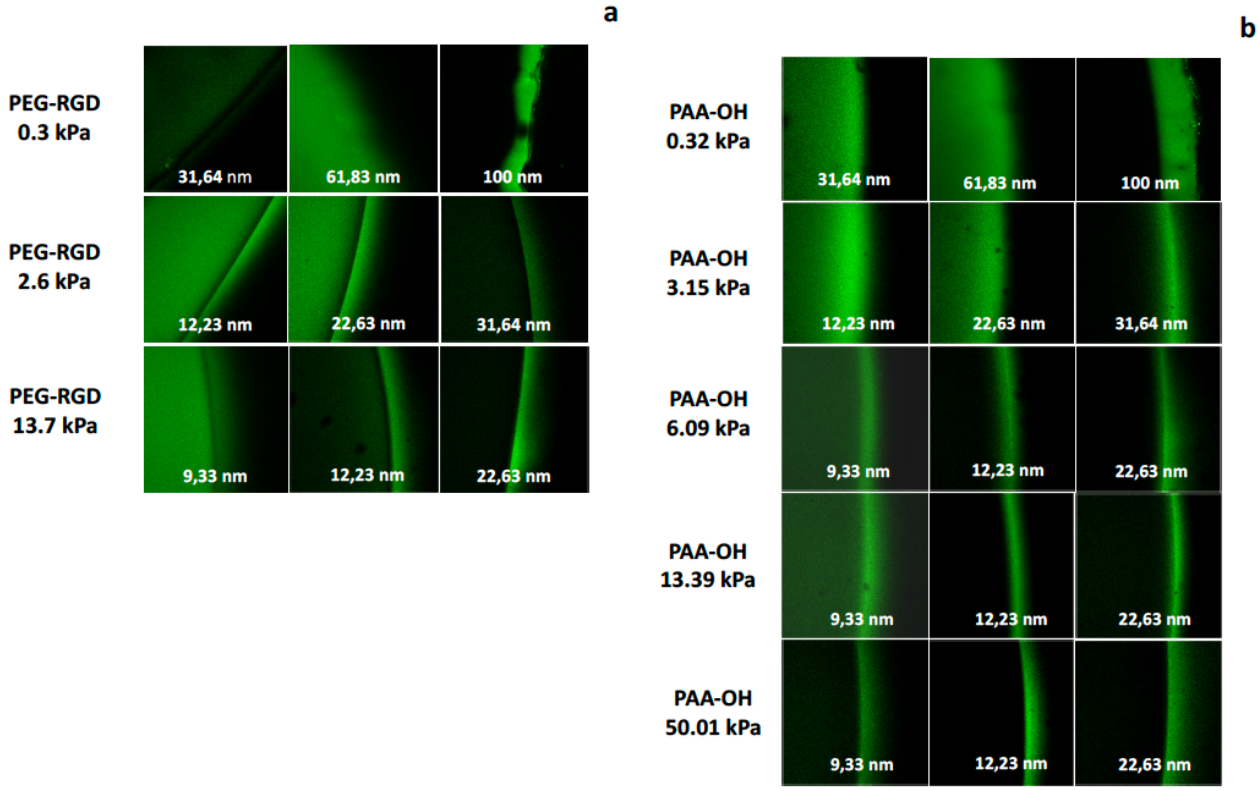


**Figure S7:** Representative confocal images of the gel-solution interface used to evaluate the cut-off values of mesh size for the reported compositions of **a)** PEG-RGD and **b)** PAA-OH. Fluorescent dextrans or nanoparticles (in green) with different molecular weights or size respectively were monitored to analyze the diffusion of molecules with defined size in the polymeric network. Diffusion was evaluated analyzing the presence of green signal in the gel after 24 hours.

**FIGURE S8**

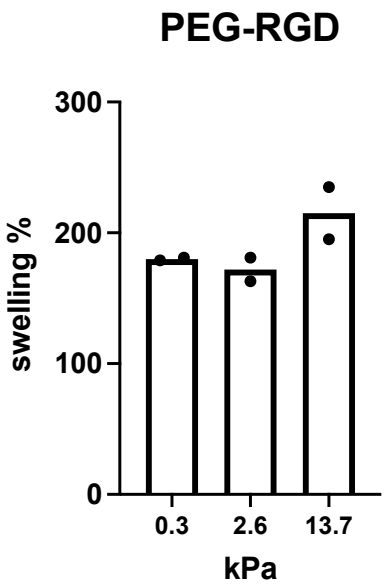


**Figure S8:** Hydrogel swelling measured using an automatic surface approaching sequence of a Netzsch lab+ rheometer with plate-plate configuration for PEG-RGD hydrogels. Samples' thicknesses were measured post-synthesis and after overnight swelling. For each composition the percentual swelling compared to the initial height is reported as the mean of two different samples. Lateral swelling is considered as negligible due to the adhesion to the underlying glass substrate.