Supporting Information for

Enhanced cell adhesion on bio-inspired hierarchically structured polyester modified with gelatin-methacrylate

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1. PDMS mold fabrication

PDMS molds were fabricated according to published protocols.[1,2] The schematic of the fabrication process is shown in Figure S1. Briefly, a PDMS mold with inverse rose petal structures (concavely curved) was formed by pouring the PDMS precursor mixed with curing agent (10:1, Sylgard 184, Dow Corning) onto the untreated fresh rose petals, followed by curing at 70ºC for 2 h. After > 10 h, the dried rose petals were gently removed from the PDMS surface. To fabricate PDMS mold with rose petal structures (convexly curved), a 10 wt% poly(vinyl alcohol) (PVA, M_W = 22,000 g/mol， Sigma-Aldrich) solution in water was poured onto the fresh rose petals and formed a film after water evaporation at ambient temperature and pressure. This PVA film possessed inverse rose petal structures and was used as template for PDMS mold fabrication, as described above.

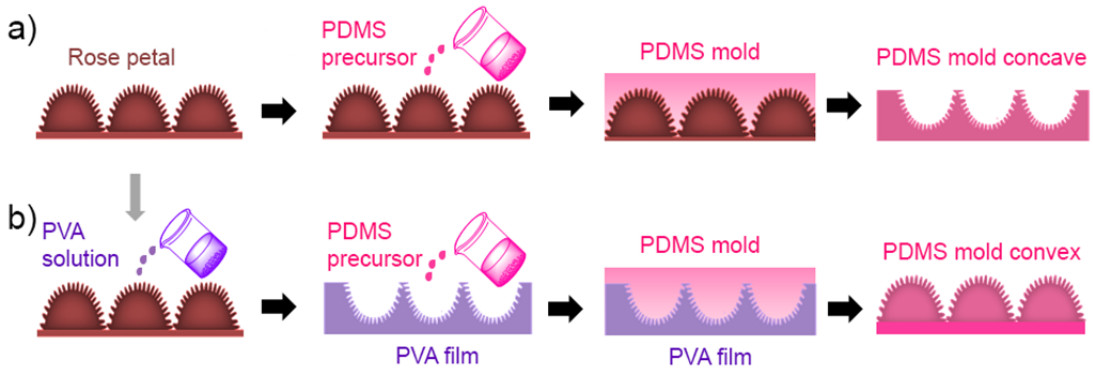
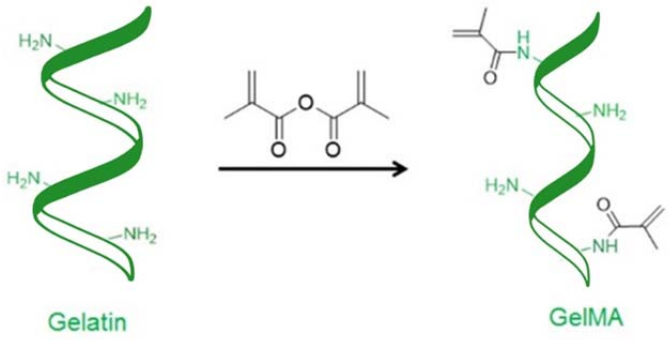


Figure S1. Schematic of PDMS mold fabrication process using the rose templates: a) fabrication of PDMS mold with concave structures and b) fabrication of PDMS mold with convex structures. Reprinted with permission from Ref. 1 and Ref. 2. Copyright 2015 and 2017 American Chemical Society.

2. GelMA synthesis and ^{1}H NMR spectra

Gelatin-methacrylate (GelMA) was synthesized according to Ref. 3, 4.



Scheme S1. Synthetic route of gelatin-Methacrylate (GelMA).

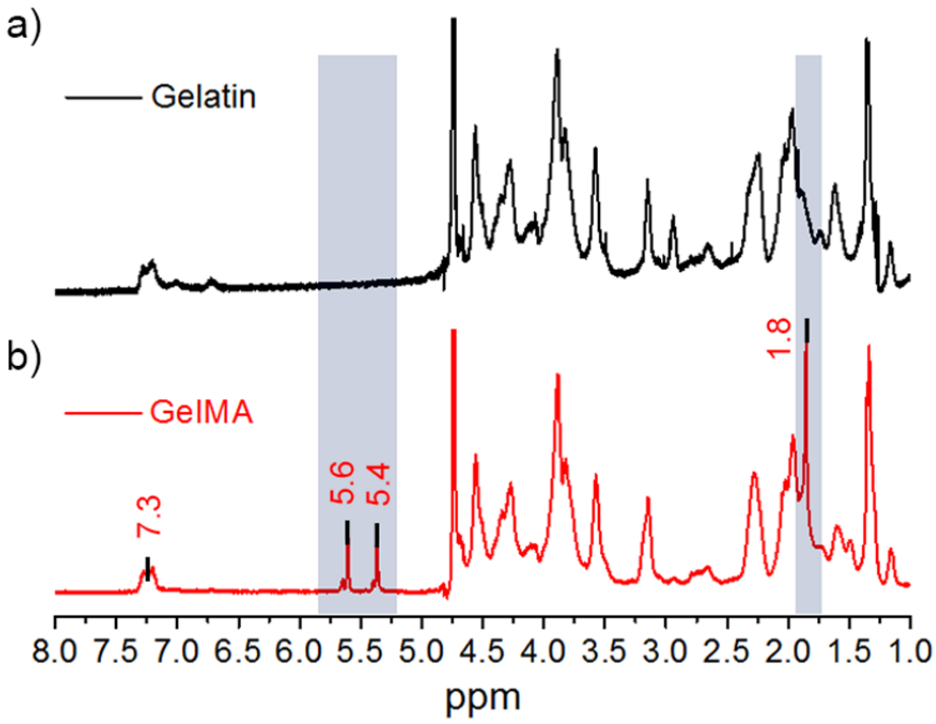


Figure S2. ^{1}H-NMR spectra of gelatin (black) and GelMA (red).

3. GelMA bulk hydrogel formation and characterization

Weighted GelMA (0.5, 1.0, and 1.5 g) was suspended to 10 mL Milli-Q water in brown glass vials, followed by shaking in an incubator (MaxQ 4000 benchtop orbital shaker, Thermo Scientific, USA) at 50°C and 200 rpm until GelMA totally dissolved to form 5, 10, 15 wt% GelMA solution. Then 0.1 g photoinitiator Irgacure 2959 was added into the vial and shaken until totally dissolved at 50°C. GelMA solution was pipped into 24 well-plate (1 mL solution per well) and subsequently exposed to UV irradiation (CL-1000 series UV crosslinker, with CL-1000L Model 365 nm UV tubes, 5 × 8 W, UVP, UK) for 15 min without any cover.

Equilibrium swelling ratio of hydrogels: The formed hydrogels were weighted and immersed into Milli-Q water at 25 °C. After 48 h, excess water was removed, and the weights of swollen hydrogel were measured after removing residue water on hydrogel surface with filter paper. The swollen hydrogels then were dried in an oven at 60°C and the weights of dried gels were recorded.

Equilibrium swelling ratio of xerogels: The formed hydrogels were dried in an oven at 60 °C and the weights of the xerogels were measured before immersing them into Milli-Q water at 25°C. After 48 h immersion, the excess water was removed and the weights of swollen hydrogels were measured after removing surface water.

Stability of GelMA hydrogels: The formed hydrogels were dried in an oven at 60 °C and the xerogels were weighted before immersing in Milli-Q water at 37°C. After 48 h, the swollen hydrogels were removed and dried in the oven at 60°C and the weights of dried gels were recorded.

4. Equilibrium swelling ratio

The equilibrium mass swelling ratio of the GelMA hydrogel was calculated as:

$$Q_H = (W_s - W_d)/W_d$$

where W_s is the weight of the gel after equilibrium swelling for 48h at 25°C and W_d is the weight of the corresponding xerogel (dried gels).

The equilibrium mass swelling ratio of the GelMA xerogel was calculated as:

$$Q_X = (W_s - W_d)/W_d$$

where W_s is the weight of the gel after equilibrium swelling for 48 h at 25 °C, and W_d is the weight of the corresponding xerogel at time 0 (swelling from dried gels).

All swelling ratio results were obtained from six samples and data are shown as the mean ± standard deviation.

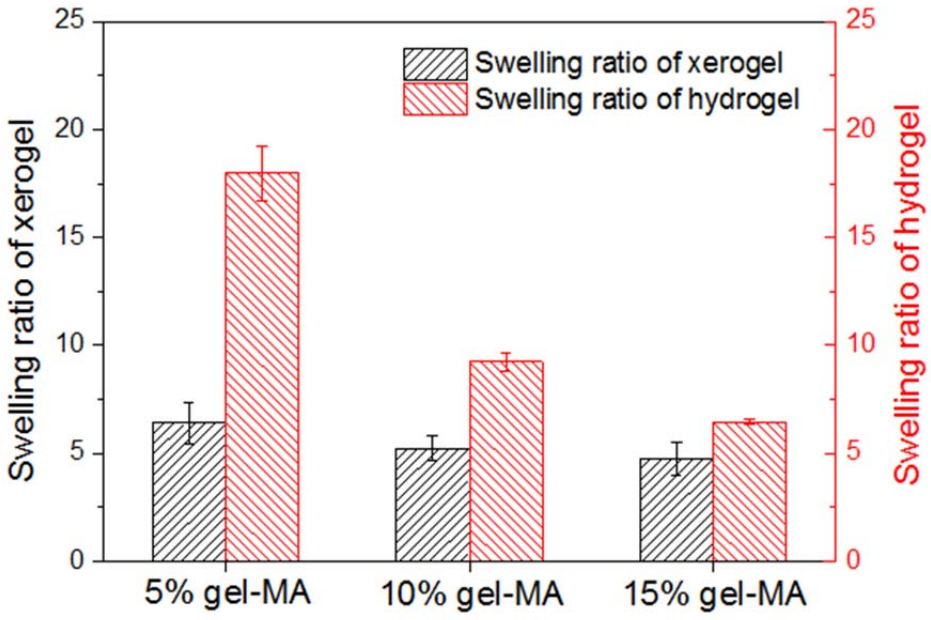


Figure S3. The equilibrium swelling ratio of 5 wt%, 10 wt%, and 15 wt% GelMA xerogels (black) and hydrogels (red) in Milli-Q water.

5. X-ray photoelectron spectroscopy (XPS)

Table S1. Elemental composition in atom% as determined by XPS of unmodified PETG and GelMA modified PETG substrates.

	XPS atomic concentrations (atom-%)		
Sample	C 1s 285 eV	O 1s 531 eV	N 1s 399 eV
Unmodified PETG	67.3	32.7	0
GelMA modified PETG	65.8	19.7	14.5

6. Water contact angle

Figure S4 Static water contact angles on GelMA modified a) flat, b) convex structured, and c) concave structured PETG substrates after 5 days storage.

7. Surface roughness of PETG substrates

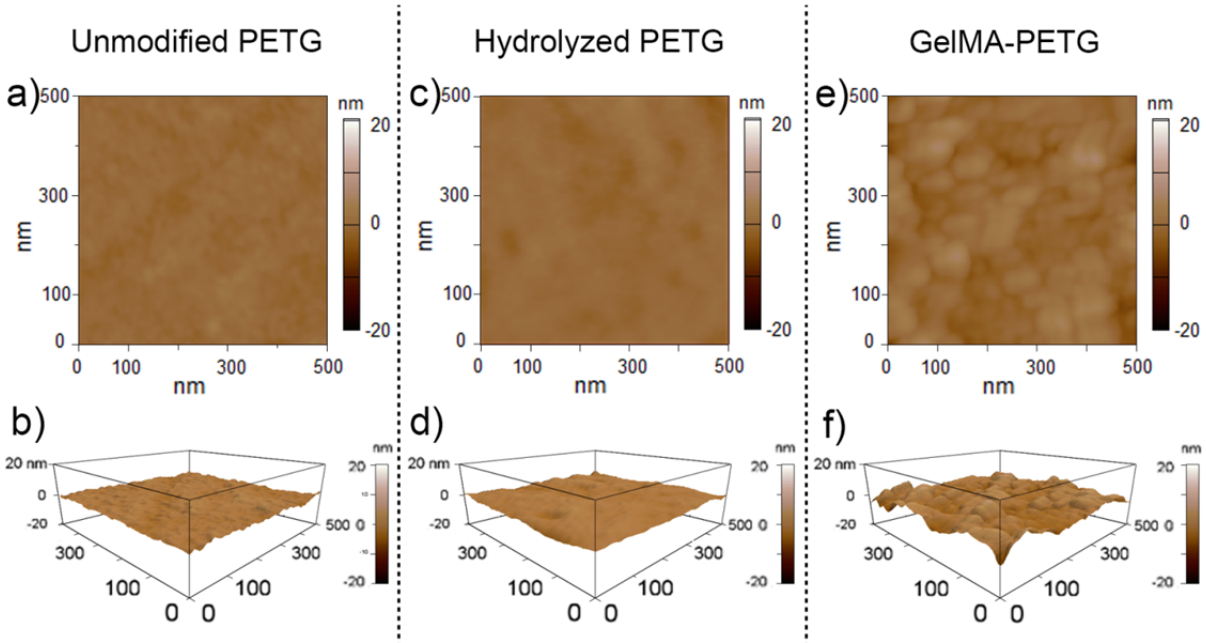


Figure S5. Two-dimensional AFM height images (upper row) and corresponding three-dimensional images (lower row) of a, b) unmodified PETG substrates; c, d) hydrolyzed PETG substrates; e, f) GelMA modified PETG substrates taken by AFM. The scan size was 500×500 nm^2.

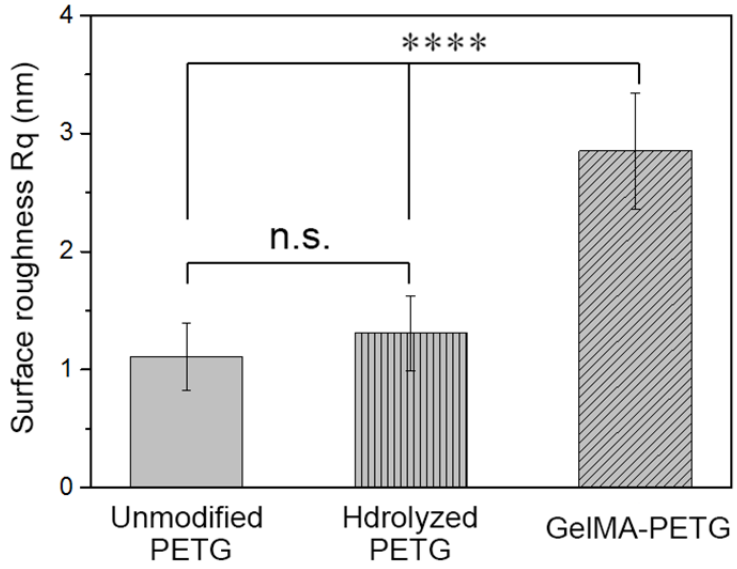


Figure S6. Comparison of surface roughness of unmodified, hydrolyzed and GelMA modified PETG substrates; **** p<0.0001 and ns stands for not significant. All the roughness values were obtained from AFM images with scan size 500 × 500 nm^2.

8. Cell Staining

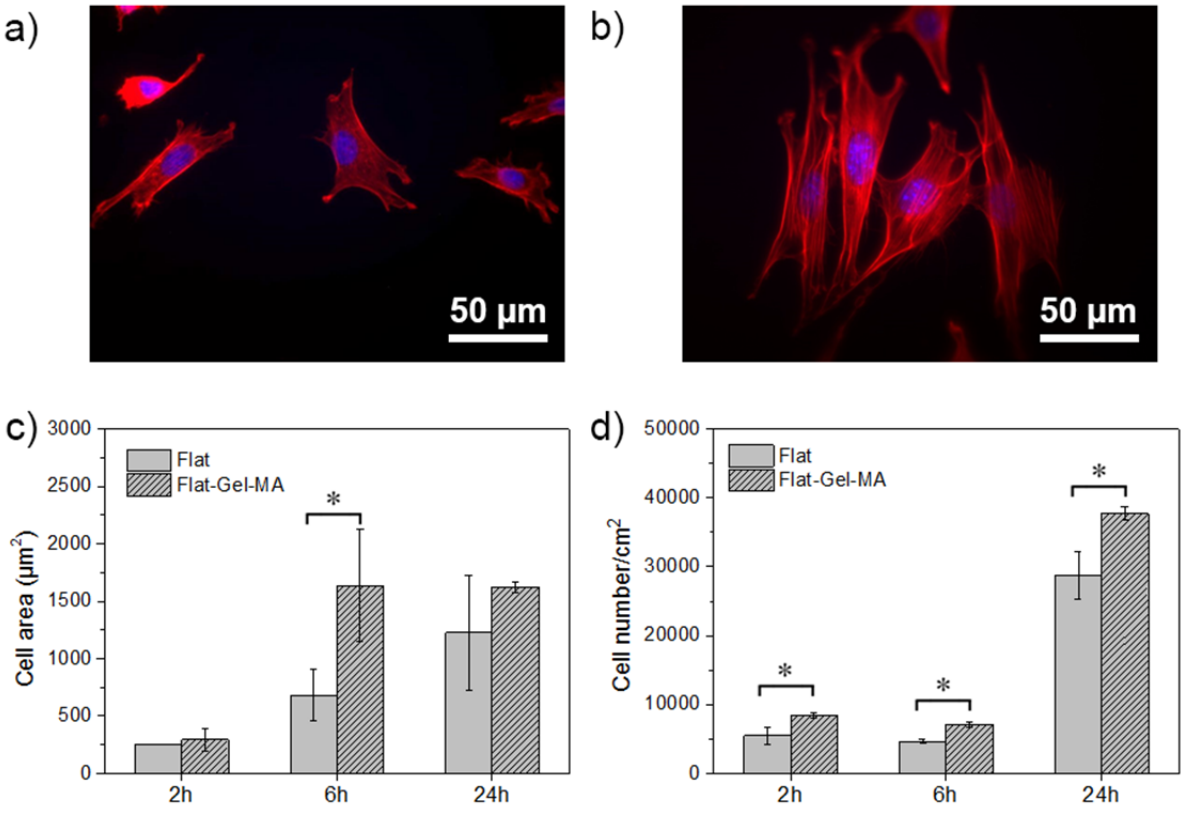


Figure S7. Immunofluorescent images of NIH-3T3 cells on flat PETG surfaces (a) without GelMA modification and (b) with GelMA modification. (c) Average cell area of single NIH-3T3 cells on flat bare PETG and GelMA modified surface, respectively. (d) Average cell number of NIH-3T3 cells on flat bare PETG and GelMA modified surface, respectively. ∗ p<0.05.

9. Cell Culture

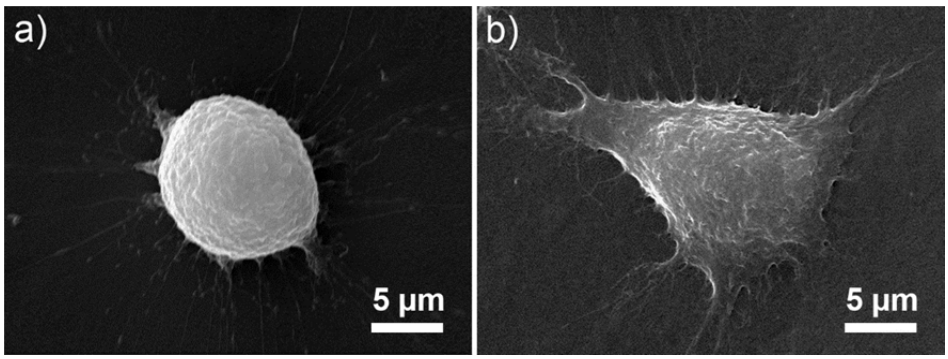


Figure S8. SEM images of NIH-3T3 cells attaching to a) unmodified and b) GelMA modified flat PETG surfaces.

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1 Dou, X.-Q.; Zhang, D.; Feng, C.; Jiang, L. Bioinspired Hierarchical Surface Structures with Tunable Wettability for Regulating Bacteria Adhesion. *ACS Nano* **2015,** *9*, 10664-10672.

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