

Supplementary Information for:

**Surface Properties of Nanostructured Bio-Active Interfaces:
Impacts of Surface Stiffness and Topography on Cell-Surface
Interactions**

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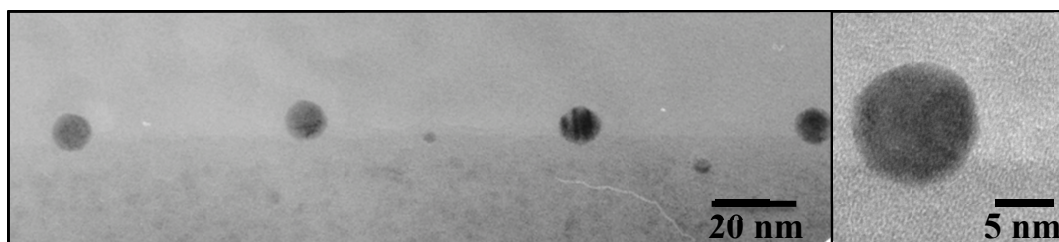


Figure S1. TEM cross-section micrographs with different resolutions of Au/Glass conformation. High-resolution image shows a gold nanoparticle partially (~25 %) embedded in the glass surface.

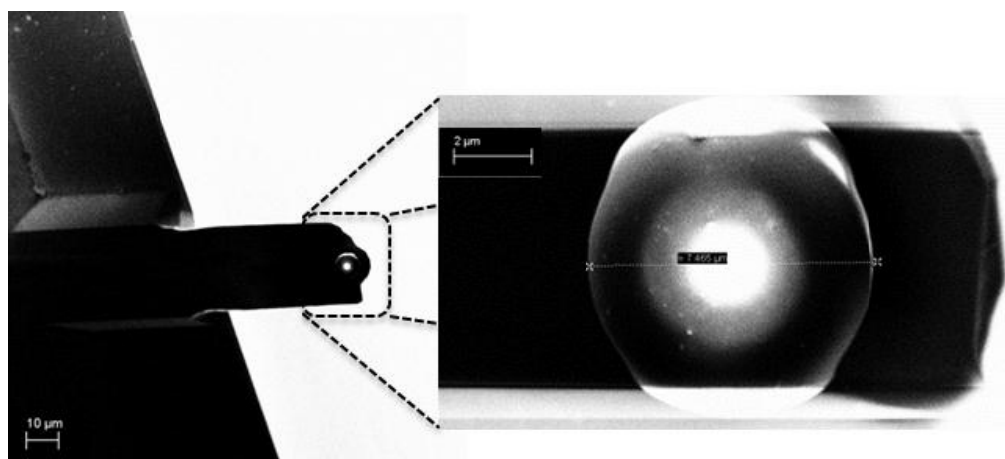


Figure S2. SEM micrographs of the cantilever used for indentation measurements with the spherical indenter of about 8 μm in diameter.

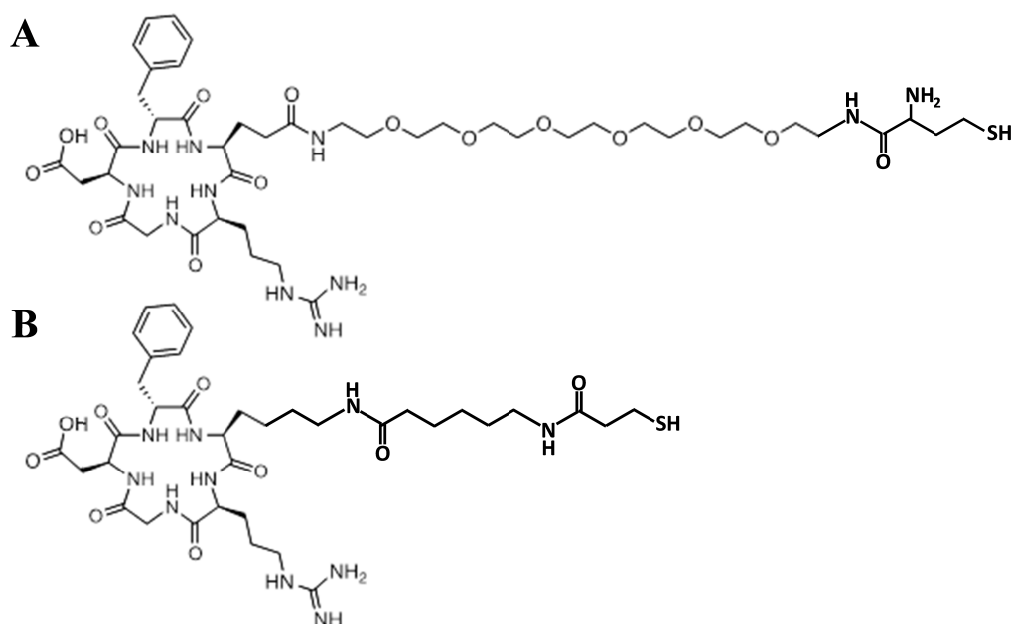


Figure S3. Molecular structure of the c(RGDfK) peptide used within this study. The cyclically arranged amino acids are arginine (R), glycine (G) and aspartate (D). A) PEG spacer (6 units) was used to breach between the peptide and the cysteine. B) Amino hexanoic (Ahx) spacer was used to breach between the peptide and the thiol.

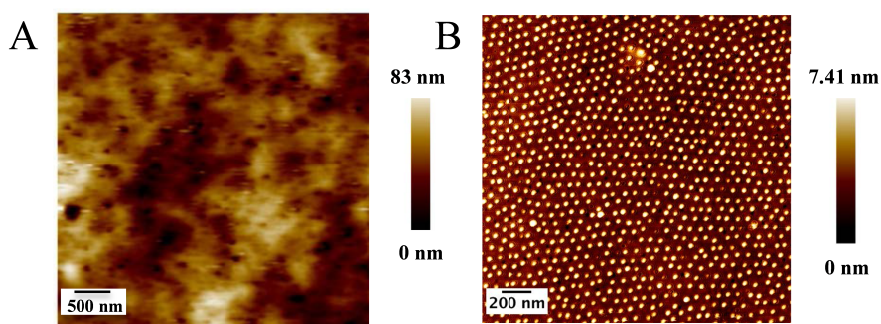


Figure S4. AFM 2D micrographs showing the rough morphology of PEG700-DA hydrogel ($R_{\text{rms}} = 17$ nm) (A) and smooth morphology of PEG-passivated glass substrate ($R_{\text{rms}} = 1.6$ nm) with interparticle spacings of 60 ± 15 nm (B).

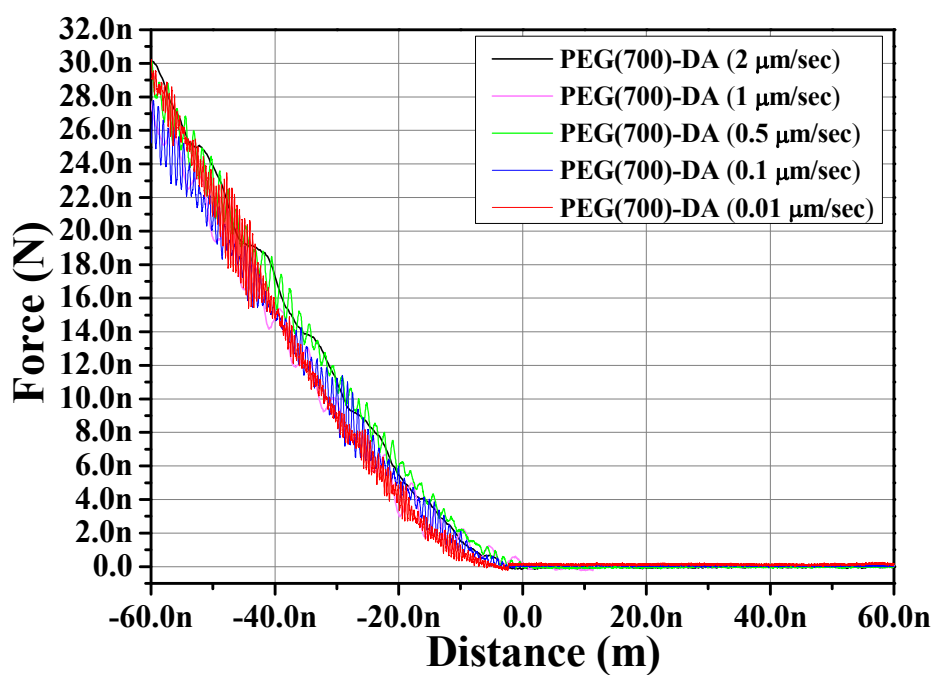


Figure S5. Representative force distance curves derived during indentation measurements on 45 nm spacing gold nanostructured PEG700-DA hydrogel substrate. The substrate was immersed in PBS solution at ambient conditions and measured with different rates of tip approach.

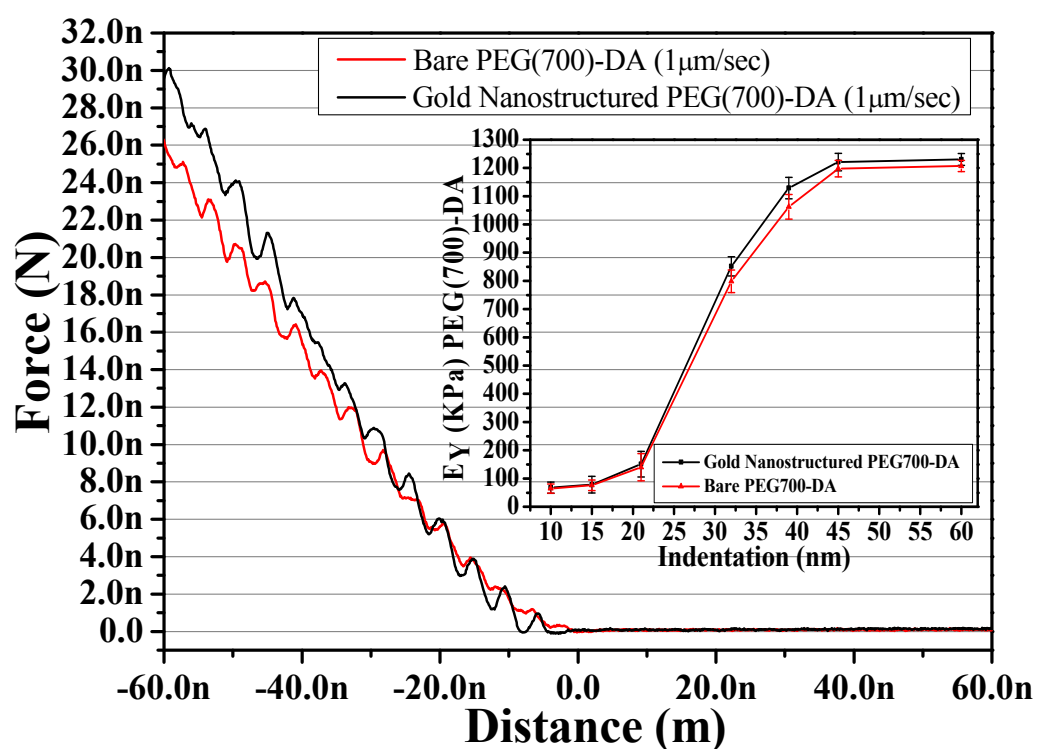


Figure S6. Representative force distance curves derived during indentation measurements on bare (red, left) and 45 nm spacing gold nanostructured (black, right) PEG700-DA hydrogel substrates. Two substrates were immersed in PBS solution at ambient conditions and measured with the same rate of tip approach (1 $\mu\text{m/sec}$). The insert shows Young's modulus dependence on indentation depth measured with different rates of tip approach on gold nanostructured PEG700-DA and bare hydrogels.

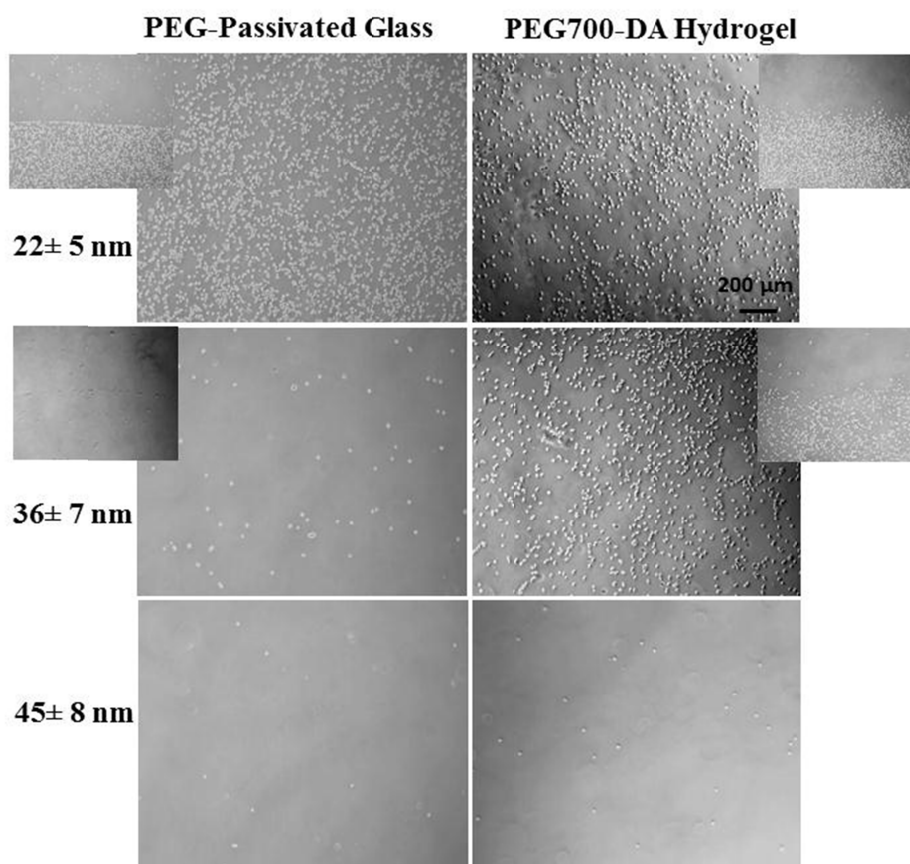


Figure S7 Phase-contrast images of KG1a cell observed at 1 h after plating on nanostructured and cRGD functionalized PEG-passivated glass (left column) and PEG700-DA hydrogel (right column) surfaces. The inserts show the surfaces consisted of a gold nanostructured area beneath the dipping line and the area with no gold nanoparticles above the dipping line (microscopic images), the cells can be seen as bright spots on a grey background.

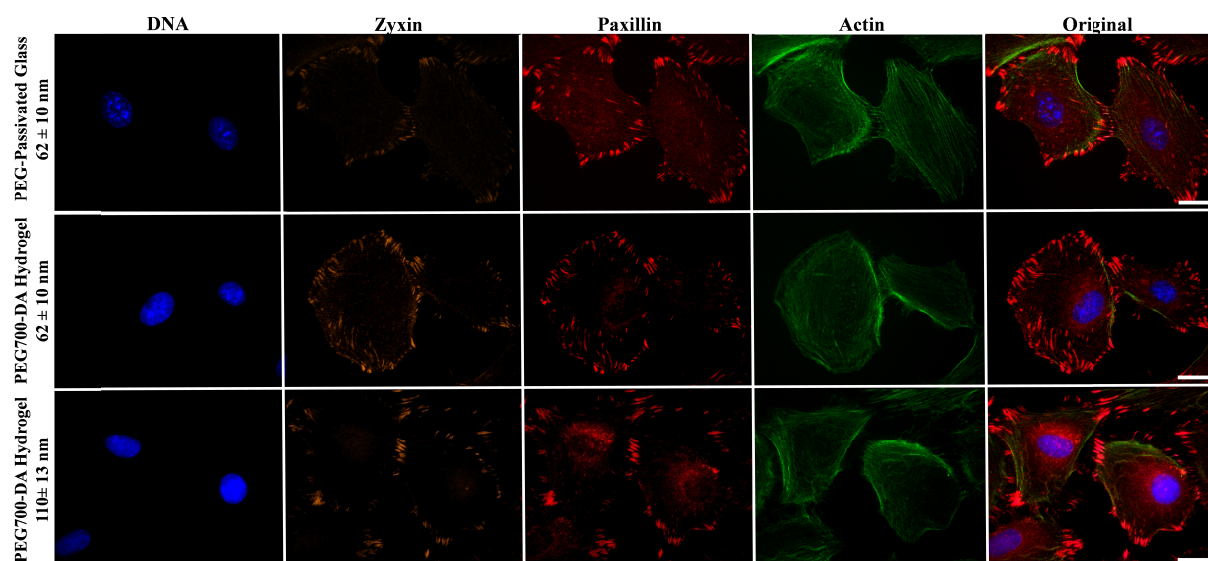


Figure S8. Immunofluorescence micrographs of fibroblasts (REF52) observed at 4 h after plating on nanostructured and cRGD functionalized PEG-passivated glass (62 nm cRGD-nanopatternes, upper row), PEG700-DA hydrogel (62 nm cRGD-nanopatternes, middle row) and PEG700-DA (110 nm cRGD-nanopatternes, lower row) surfaces. REF52 cells were fixed and immunostained with primary antibodies against zyxin and paxillin, followed by Alexa 594- and 647-conjugated secondary antibodies, respectively. Filamentous actin and nuclei were labeled with Alexa 488-conjugated phalloidin and DAPI, respectively. Scale bars: 30 μ m.