Supporting Information for

Surface-Adhesive and Osteogenic Self-Assembled Peptide Nanofibers for Bioinspired Functionalization of Titanium Surfaces

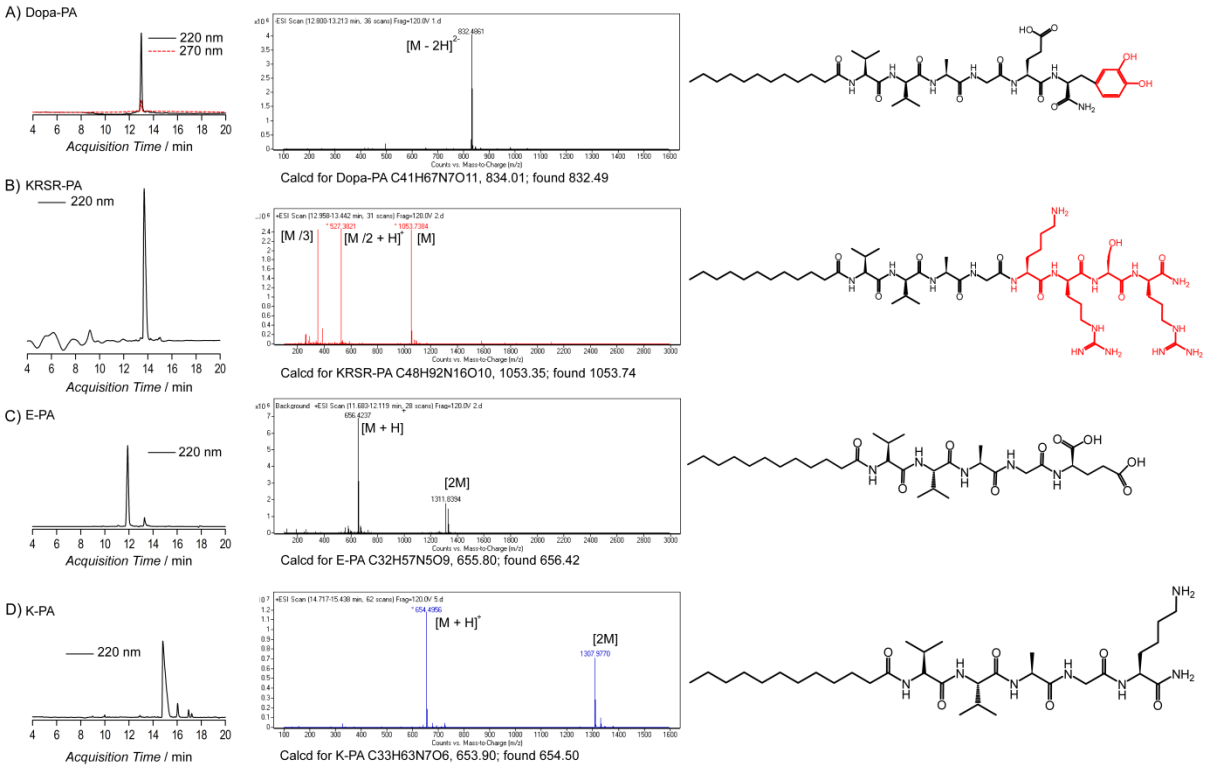

Figure S1. All PA batches were synthesized and used with more than 90% purity. Purity levels were assessed according to amide content. Each synthesis was characterized with analytical HPLC equipped with mass spectrometer for Dopa-PA, KRSR-PA, E-PA and K-PA, respectively. E-PA was synthesized as a control PA molecule for Dopa-PA and K-PA was synthesized as a control molecule for KRSR-PA. All PA molecules were designed by utilizing similar principles. The aliphatic hydrophobic tail, lauric acid, drives the hydrophobic collapse and hence was attached adjacent to the β-sheet-driving hydrophobic amino acids, which are located in the order of Val-Val-Ala-Gly, from C to N terminal. These amino acids were incorporated in the order of increasing hydrophilicity as the design requires. The self-assembly mechanism and the spatial arrangement of the ligands on the fibers enabled presentation of more than one functional unit at the same time.

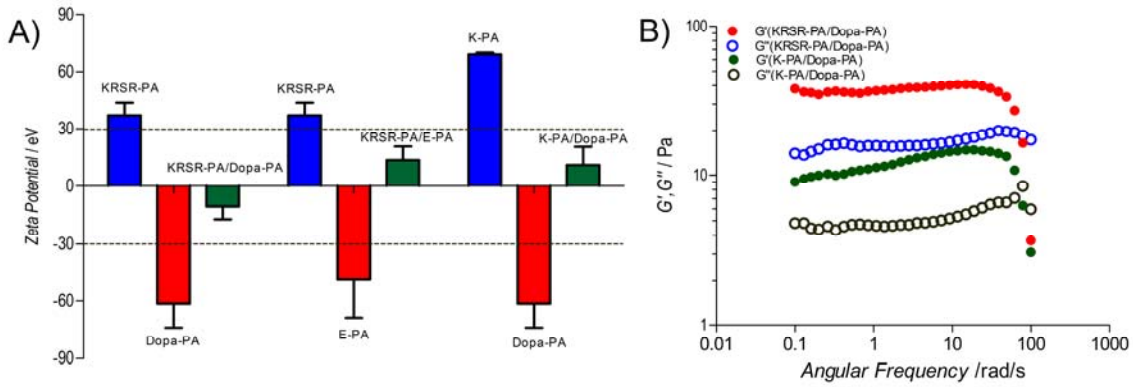

Figure S2. A) Zeta-potential measurements of individual PAs and their mixture as used in the article. B) Rheology measurements of PAs used in in vitro experiments to show the formation of a network that has soft mechanical properties, which mimicked native ECM.

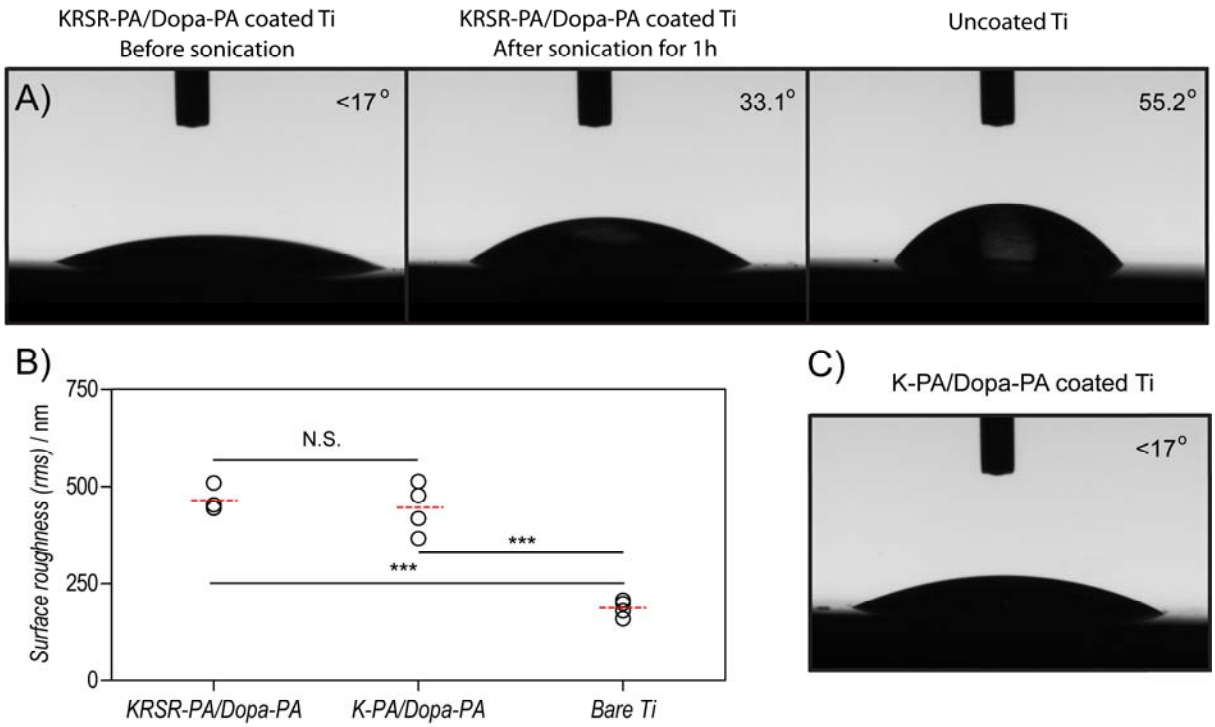

Figure S3. A) Contact angles of titanium substrates as bare, KRSR-PA/Dopa-PA coated and after 1 h sonication following KRSR-PA/Dopa-PA coating. B) Surface roughness (in terms of root mean square, or rms) of PA nanofibers and bare titanium surface used in in vitro experiments. C) Contact angle of K-PA/Dopa-PA coated titanium surface.***p<0.0001, N.S., no significance.

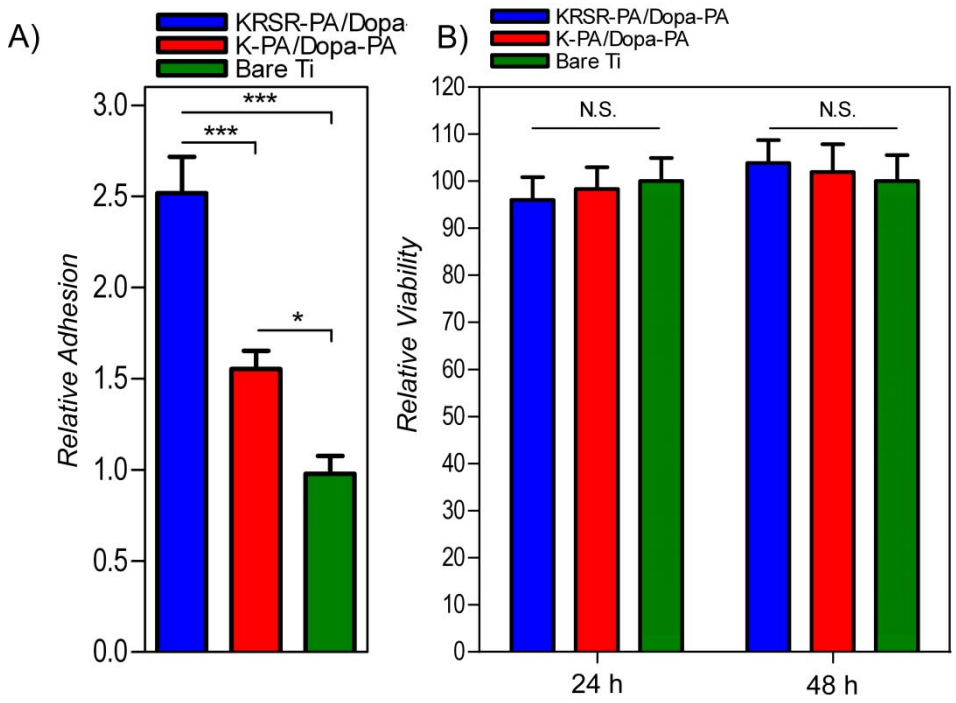

Figure S4. Adhesion (A) and viability (B) of MC3T3-E1 cells on functionalized titanium surfaces.*p<0.05, ***p<0.0001, N.S., no significance.

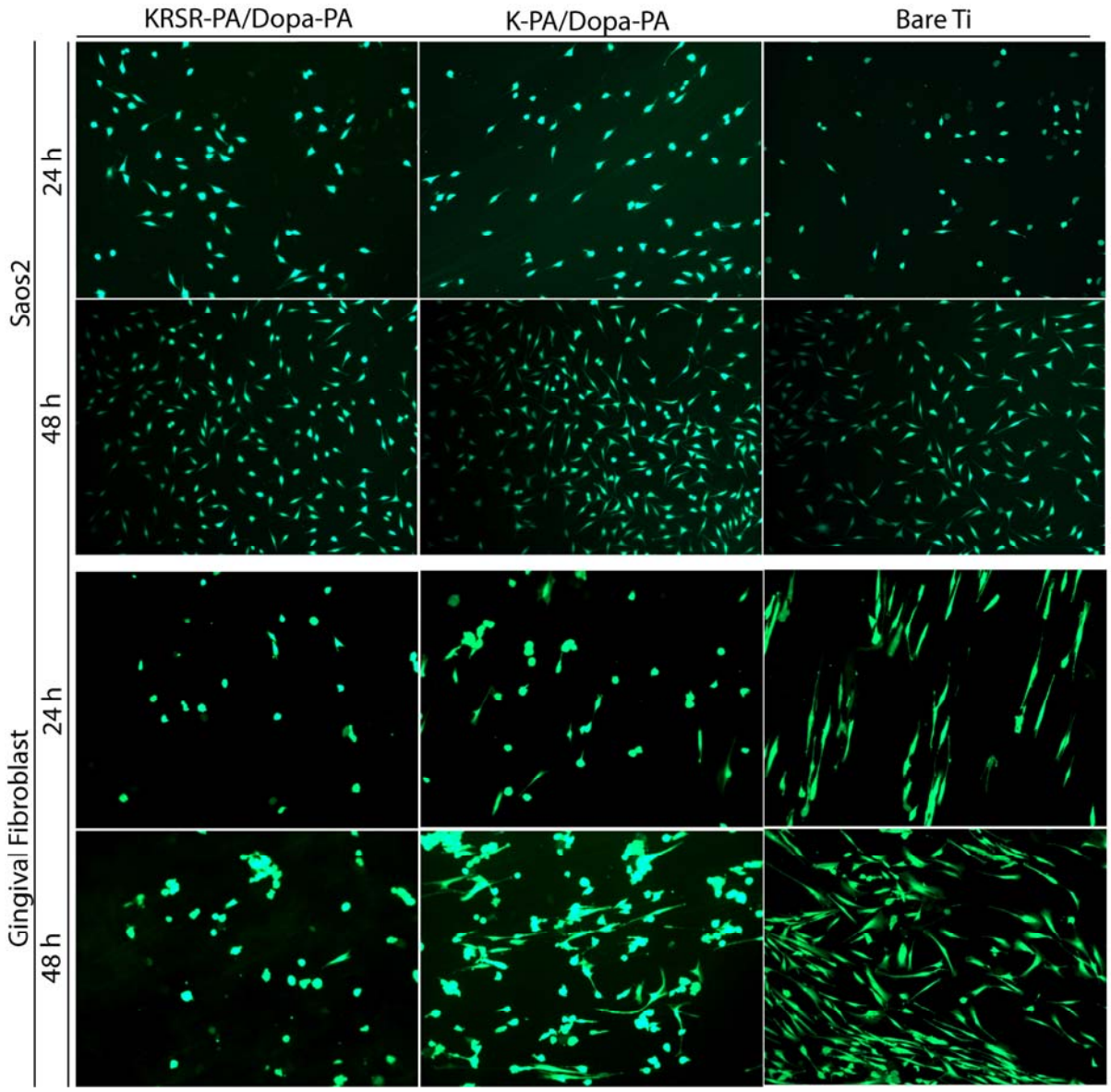

Figure S5. Representative Calcein-AM stained micrographs (10X) of Saos2 and HGF cells at 24 and 48 h on PA coated and bare titanium surfaces.

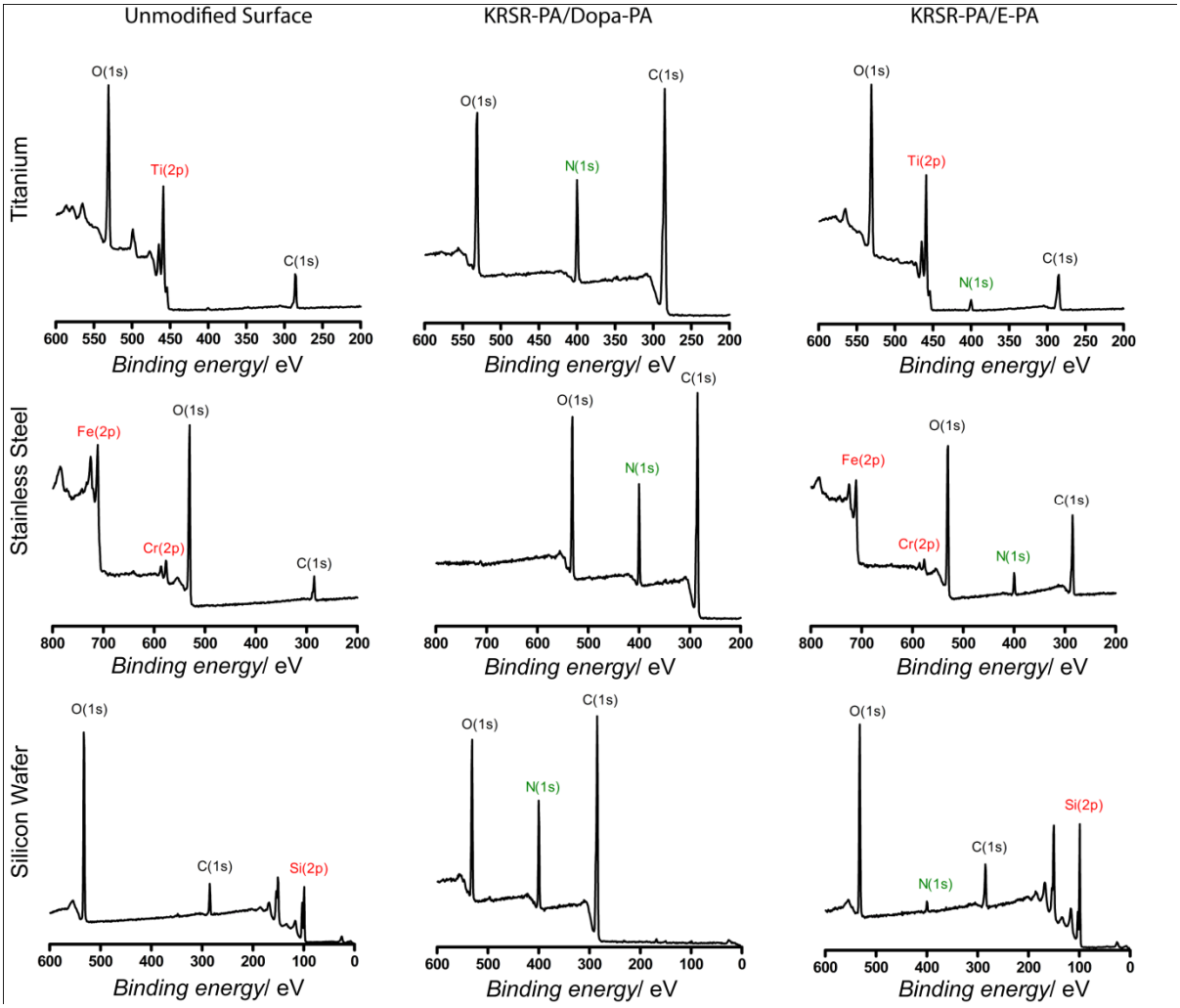

Figure S6. XPS spectra of functionalized titanium, stainless steel, and silicon surfaces with KRSR-PA/Dopa-PA nanofibers.

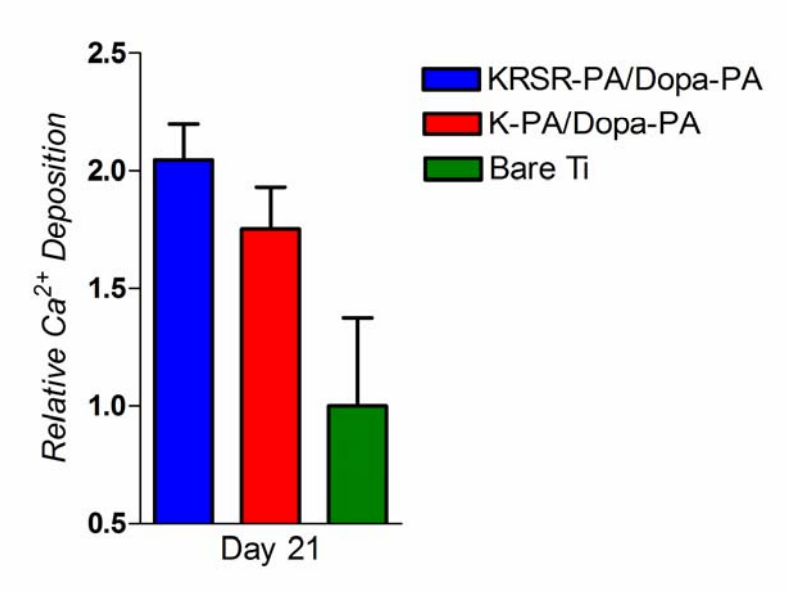

Figure S7. Quantification of relative calcium deposition on the matrix on day 21.

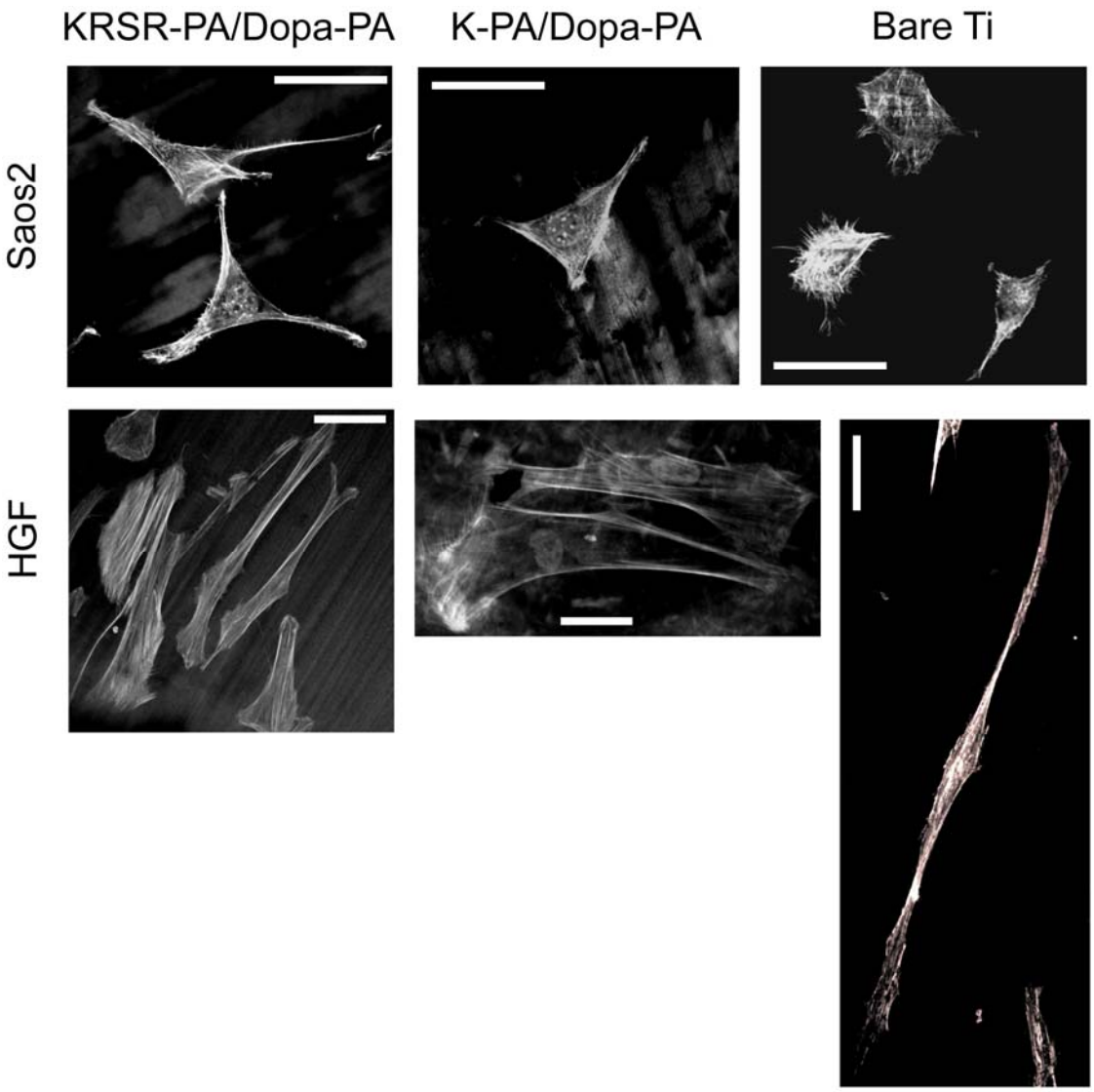

Figure S8. Representative high magnification confocal images of the cells at 24 h. Actin microfilaments and nuclei of cells were stained with TRITC-phalloidin and TO-PRO®, respectively. Scale bars show 50 μm.

Assignment of FT-IR spectra peaks on KRSR-PA/Dopa-PA coated titanium surface

(Figure 3D in the Manuscript)

FT-IR spectra in the range of 1100 and 1700 cm^{-1} for PA nanofibers adsorbed on stainless steel surface provided information to characterize the presence of Dopa on adsorbed PA nanofibers. In peptide structures, amide I is the most intense absorption band, which is characterized by absorption in the range of 1600-1700 cm^{-1}. Absorption at this range is governed by the stretching vibrations of the carbonyl (C=O) and amide (C-N) groups. The exact localization of the band centre is related to the secondary order of the structure. In our experiment, the amide I band was found to be centered to 1639 cm^{-1}. Absorption at this wave number had been previously reported to indicate β-sheet-rich secondary order. This observation is consistent with the self-assembly-driven nanofiber formation by means of β-sheets between adjacent micelles and with the results of circular dichroism measurements. Amide II is characterized by absorption in the range of 1490 to 1600 cm^{-1}; 1540 cm^{-1} in our construct. It mainly derives from N-H bending and C-N stretching vibrations. The difference in position of the amide II band is believed to result from differences in experimental conditions. Absorption around the band centered at 1455 cm^{-1} is expected for the vibration of substituted aromatics. In previous studies, similar bands were characterized for adsorbed *mefp-1* protein in this region and were attributed to the presence of Dopa. The band centered at 1382 cm^{-1} is due to the alkane -C-H bending that is found in peptide backbone. The band that is centered at 1236 cm^{-1} was previously associated with C-O stretching vibrations of the catecholic side chains of Dopa. The broad band spanning 960 and 1120 cm^{-1} is reported to arise from the carboxylic and phenol residues due to the presence of glutamic acid and Dopa.