

Supporting information for

Synergistic Anti-fouling and Bactericidal Poly(ether ether ketone) Surfaces via a One-step Photomodification

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Synthetic procedures

4-azidophenyl methacrylate

Briefly, TEA (5.8 ml, 42 mmol) was added to a solution of 4-azidophenol (4.8 g, 35 mmol) in DCM (50 ml) at 0 °C and the mixture was stirred for 5 min. Subsequently a solution of methacryloyl chloride (3.8 ml, 39 mmol) in DCM (5 ml) was added dropwise, the reaction mixture was allowed to warm to room temperature and stirred overnight. After the reaction, the mixture was filtered over a fritted glass filter to remove the TEA salt and the organic phase was washed with brine, dried with Na₂SO₄, filtered and concentrated. The product was purified by column chromatography on silica gel with DCM.

4-APM was obtained as a yellow solid. Yield: 5.1 g (60 %). ¹H NMR (300 MHz, CDCl₃, δ): 7.13 (d, 2H, phenyl protons), 7.05 (d, 2H, phenyl protons), 6.35 (s, 1H, =CH), 5.77 (s, 1H, =CH), 2.06 (s, 3H, C(CH₃)=CH).

(PEG-XX)₂-ABCPA macro-initiators

(PEG-5K)₂-ABCPA was synthesized following a previously published procedure [1]. Briefly, ABCPA (0.11 g, 0.40 mmol), mPEG5K (4.0 g, 0.80 mmol) and DPTS (38 mg, 0.13 mmol) were dissolved in 22 ml of a mixture of DCM and dry DMF (10/1 v/v). After cooling the solution in an ice bath, a solution of DCC (0.25 g, 1.2 mmol) in 5 ml DCM was added dropwise under stirring. The mixture was allowed to warm to room temperature and stirred overnight in an argon atmosphere. The reaction mixture was filtered, concentrated and precipitated in cold diethyl ether. (PEG-5K)₂-ABCPA was obtained by filtration and dried in vacuo to give a white powder. Yield: 3.4 g (85 %). ¹H NMR (300 MHz, CDCl₃, δ): 4.25 (m, 4H, terminal PEG protons), 3.63 (m, 1020H, PEG protons), 3.37 (s, 6H, CH₃-O-CH₂-CH₂), 2.30 - 2.64 (br, 8H, C-CH₂-CH₂-C=O), 1.66 and 1.72 (s, 6H, C-CH₃).

(PEG-2K)₂-ABCPA and (PEG-12K)₂-ABCPA were prepared following analogous procedures, starting from mPEG2K and mPEG12K, respectively.

(PEG-XK)-P(HPMA) diblock copolymers

(PEG-5K)-P(HPMA) was synthesized from (PEG-5K)₂-ABCPA following a previously reported procedure [1]. (PEG-5K)₂-ABCPA (0.80 g, 7.8×10^{-2} mmol) and HPMA (0.80 g, 5.6 mmol) were dissolved in 10 ml of acetonitrile. The solution was stirred at 70 °C overnight under reflux in an argon atmosphere. After removal of the acetonitrile by rotary evaporation, the product was dissolved in DCM and precipitated in cold diethyl ether. (PEG-5K)-P(HPMA) was obtained by filtration and dried overnight in vacuo to give a white powder. Yield: 1.3 g (83 %). ¹H NMR (300 MHz, D₂O, δ): 4.02 (br, 38H, NH-CH₂-CH(CH₃)-OH), 3.80 (m, 510H, PEG protons), 3.48 (s, 3H, CH₃-O-CH₂-CH₂), 3.1 - 3.4 (br, 76H, NH-CH₂-CH), 1.7 - 2.2 (br, 76H, backbone methylene protons), 1.29 (br, 114H, NH-CH₂-CH(CH₃)-OH), 1.0 - 1.2 (br, 114H, backbone methyl protons). SEC: M_n 8800 g mol⁻¹, D 1.34.

(PEG-2K)-P(HPMA) and (PEG-12K)-P(HPMA) were prepared following analogous procedures, starting from (PEG-2K)₂-ABCPA and (PEG-12K)₂-ABCPA, respectively.

(PEG-XK)-P(HPMA-N₃) diblock copolymers

For the synthesis of (PEG-5K)-P(HPMA-N₃), (PEG-5K)-P(HPMA) (0.75 g, 8.1×10^{-2} mmol), 4-azidobenzoic acid (0.43 g, 2.6 mmol) and DMAP (38 mg, 0.31 mmol) were dissolved in 10 ml dry DMF. After cooling the solution in an ice bath, a solution of DCC (0.54 g, 2.6 mmol) in 5 ml DMF was added dropwise under stirring. The mixture was allowed to warm to room temperature and stirred overnight in an argon atmosphere. The reaction mixture was filtered, concentrated and precipitated in cold diethyl ether. (PEG-5K)-P(HPMA-N₃) was obtained by filtration and dried in vacuo to give a light brown powder. Yield: 0.73 g (62 %).

^1H NMR (300 MHz, DMSO- d_6 , δ): 7.96 (br, 38H, phenyl protons), 7.21 (br, 38H, phenyl protons), 5.00 (br, 19H, NH-CH₂-CH(CH₃)-O-C=O), 4.72 (br, 19H, CH(CH₃)-OH), 3.51 (m, 510H, PEG protons), 3.24 (s, 3H, CH₃-O-CH₂-CH₂), 2.90 (br, 76H, NH-CH₂-CH), 1.4 - 2.0 (br, 76H, backbone methylene protons), 0.99 (br, 114H, NH-CH₂-CH(CH₃)-O), 0.7 - 0.9 (br, 114H, backbone methyl protons).

(PEG-2K)-P(HPMA-N₃) and (PEG-12K)-P(HPMA-N₃) were prepared following analogous procedures, starting from (PEG-2K)-P(HPMA) and (PEG-12K)-P(HPMA), respectively.

Characterization of functionalized PEEK surfaces

FTIR spectra were recorded using a Perkin-Elmer Spectrum 100 FTIR spectrometer.

Static water contact angles were obtained via the sessile drop method. A PEEK disc was placed in a system consisting of a sample holder, a light source and a Dataphysics OCAH200 CDD camera. Subsequently one drop of distilled water (approximately 50 μl) was placed on the PEEK disc using a pipette. The drop profile was captured with the camera and analyzed with ImageJ 1.51 software employing the DropSnake plug-in. Three sample points were collected for each PEEK sample.

XPS analyses were performed on an ESCALAB 250 photoelectron spectrometer (Thermo Electron). The excitation source was the Al K α X-ray at 1486.6 eV. The spectra were collected from a surface with a diameter of 500 μm and calibrated to the binding energy of the C-C component of the C_{1s} peak to 284.8 eV.

Atomic Force Microscopy (AFM) studies were performed using a Nanoman atomic force microscope (Bruker Metrology, Santa Barbara, USA) equipped with a closed loop and run by Nanoscope 5 software (Bruker Instruments). Using an NCL cantilever (Nanosensors, Neuchatel, Switzerland) images were recorded in tapping mode under ambient conditions. Images were processed using Gwyddion 2.50 software.

Evaluation of cytotoxicity via extraction method (ISO 10993-5)

Extract preparation

PEEK samples ($n = 3$) were autoclaved and Dulbecco's modified Eagle's medium (Sigma, 4.5 g l⁻¹ *D*-glucose) supplemented with 5 % fetal bovine serum (Gibco), 2 mM *L*-glutamine (Gibco), 100 units ml⁻¹ penicillin (Sigma) and 1 % streptomycin (Sigma), named extraction vehicle, was added to achieve an extraction ratio of 3 cm² ml⁻¹ (ISO 10993-12). Extraction was performed for 72 h at 37 °C under stirring. The same protocol was used to prepare extracts from standard reference materials (ISO 10993-5, $n = 3$). In this study, high density polyethylene film and 0.25 % zinc dibutyldithiocarbamate (ZDBC) polyurethane film (both from Hatano Research Institute, Japan) were used as negative and positive control, respectively.

Test on L929 cells

L929 fibroblasts, tested to be free of mycoplasma, were seeded at $1 \cdot 10^4$ cells per well (96-well plate) and allowed to adhere overnight. Subsequently extracts were added to the L929 cell monolayer. The following concentrations of the test item extracts were tested: 25 %, 50 %, 75 % and 100 % (*v/v*). For the negative control only the 100 % extract was tested. Dilutions were prepared using the extraction vehicle. The cell viability was assessed after 24 h incubation at 37° C under humidified atmosphere with 5 % CO₂ using the CellTiter-Glo luminescence assay (Promega) and the lactate dehydrogenase activity assay (LDH assay, Pierce). Briefly, for the CellTiter-Glo luminescence assay, CellTiter-Glo reagent was added in each well with a volume equal to the volume initially present in each cell culture well. After 10 min incubation at room temperature, luminescence was recorded on a BMG Labtech CLARIOstar® microplate-reader. The luminescent signal is proportional to the amount of ATP present in cell medium after cell lysis and hence to the number of cells in the well. For the LDH assay, equal amounts of cell supernatant and LDH reaction mixture were mixed and incubated for 30 min at room

temperature. Subsequently, the absorbance was read at 490 nm using a BMG Labtech CLARIOstar® microplate-reader.

Reference

1. Neradovic, D.; Van Nostrum, C. F.; Hennink, W. E., Thermoresponsive polymeric micelles with controlled instability based on hydrolytically sensitive N-isopropylacrylamide copolymers. *Macromolecules* 2001, 34, (22), 7589-7591.

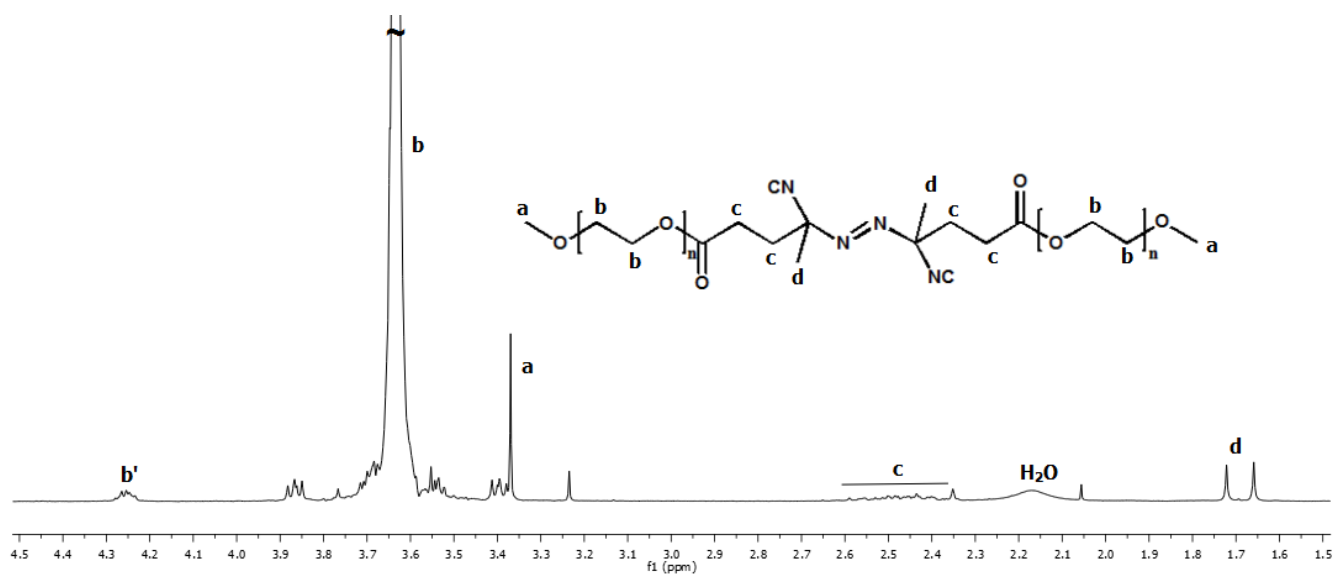


Figure S1. ¹H NMR spectrum of (PEG-5K)₂-ABCPA macro-initiator. Peak **b'** corresponds to terminal PEG protons. Solvent: CDCl₃.

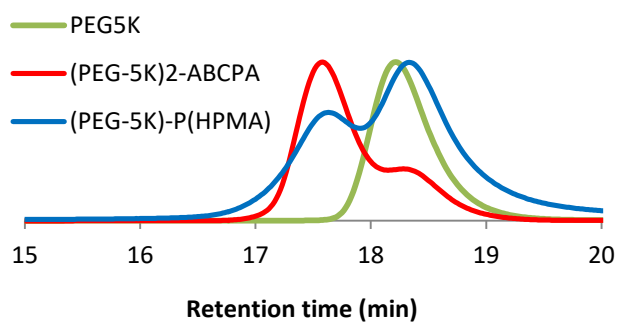


Figure S2. Normalized SEC traces of PEG-5K, (PEG-5K)₂-ABCPA and (PEG-5K)-P(HPMA).

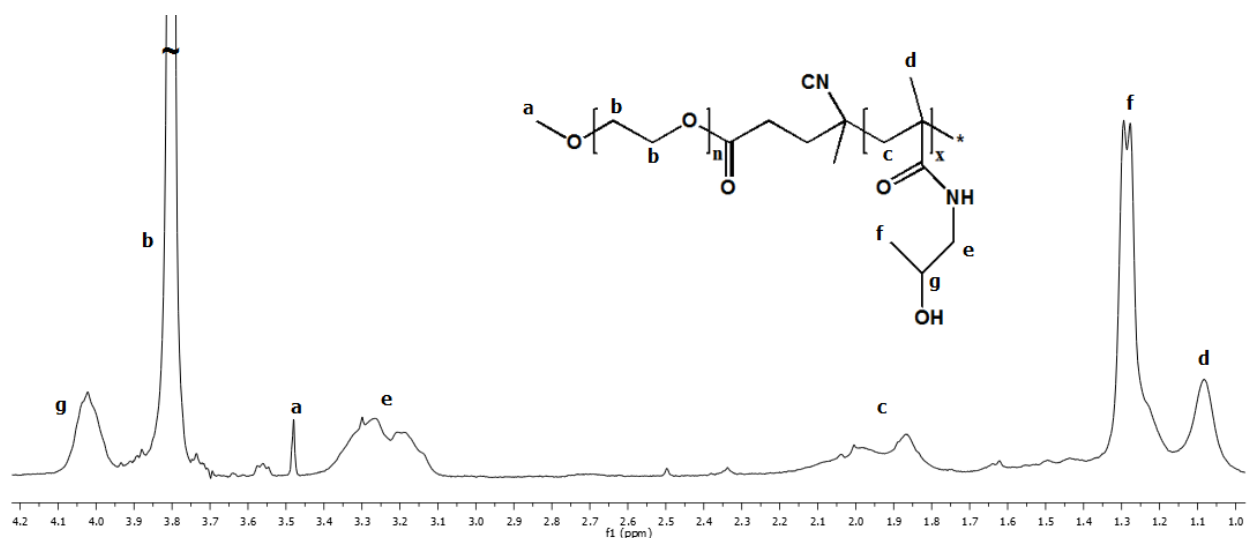


Figure S3. ^1H NMR spectrum of (PEG-5K)-P(HPMA) block copolymer. Unassigned peaks correspond to P(HPMA) backbone protons. Solvent: D_2O .

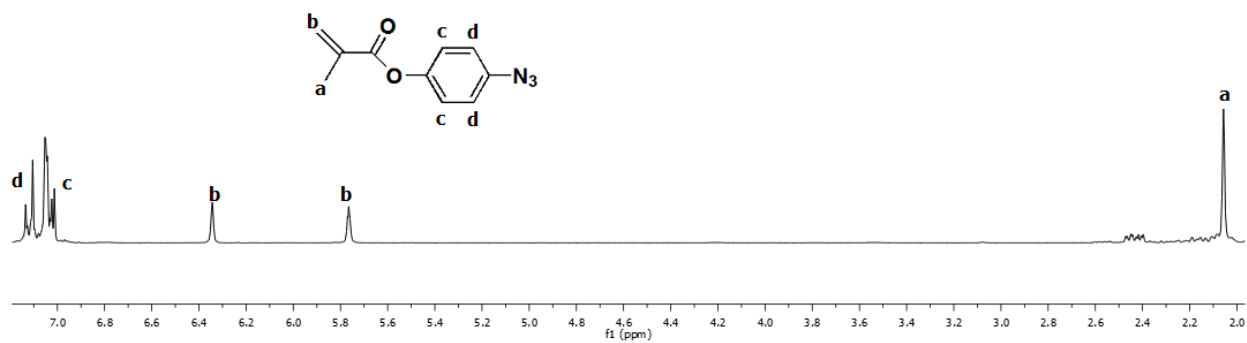


Figure S4. ¹H NMR spectrum of 4-azidophenyl methacrylate (4-APM). Solvent: CDCl₃.

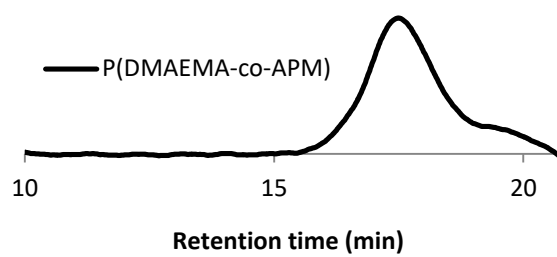


Figure S5. SEC trace of P(DMAEMA-co-APM).

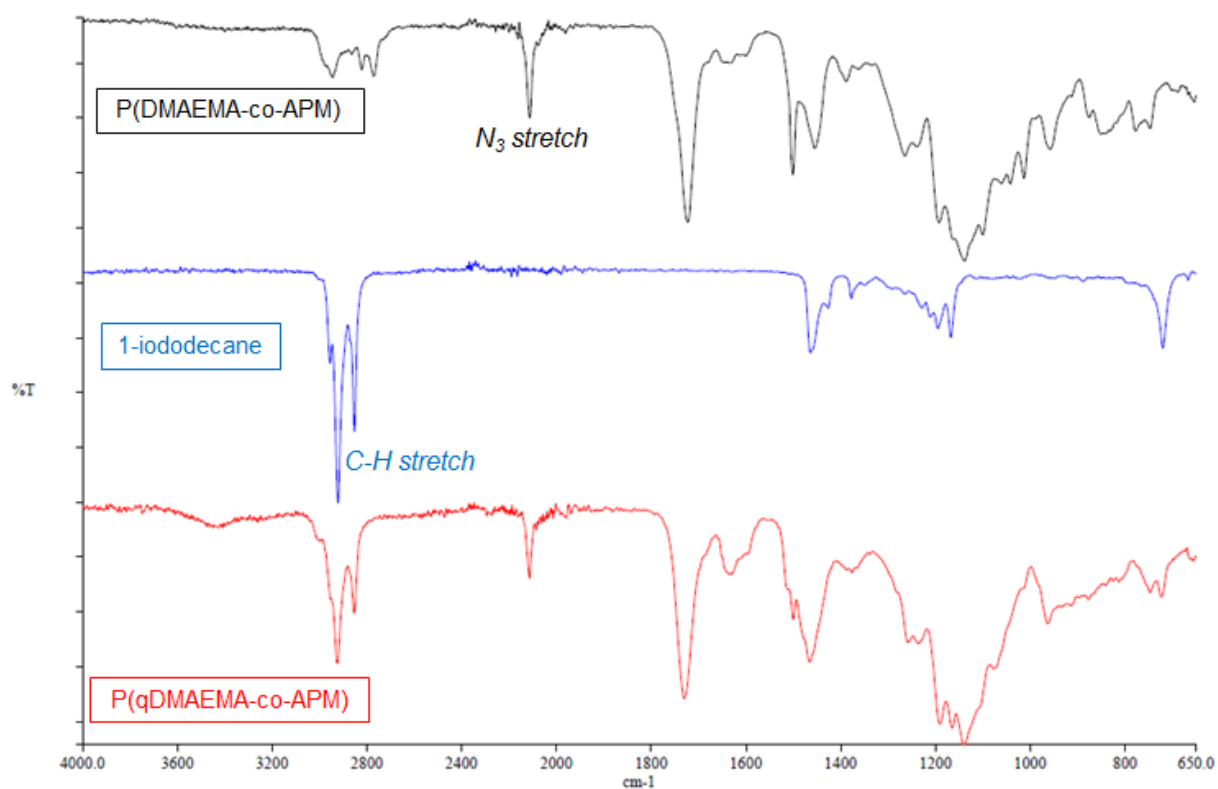


Figure S6. FTIR spectra of P(DMAEMA-co-APM), 1-iododecane and P(qDMAEMA-co-APM).

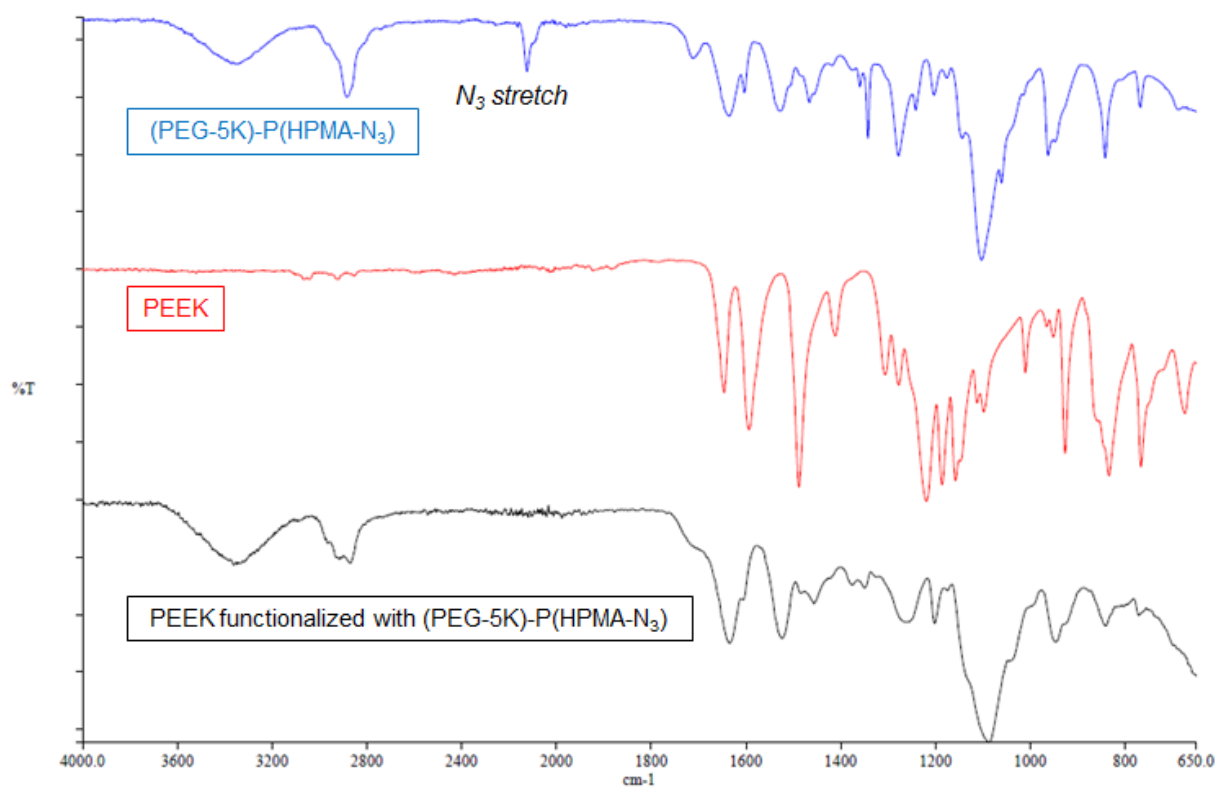


Figure S7. FTIR spectra of (PEG-5K)-P(HPMA-N₃), PEEK, and PEEK functionalized with (PEG-5K)-P(HPMA-N₃).

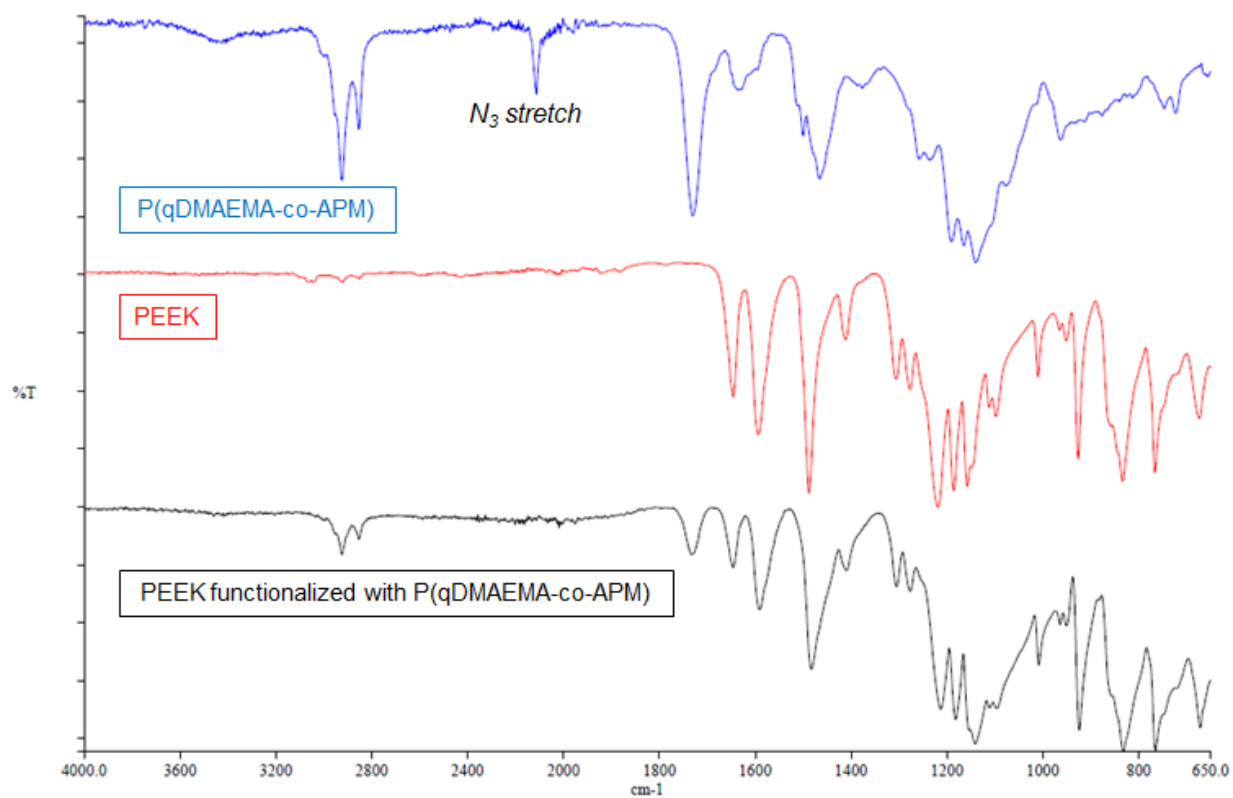


Figure S8. FTIR spectra of P(qDMAEMA-co-APM), PEEK and PEEK functionalized with P(qDMAEMA-co-APM).

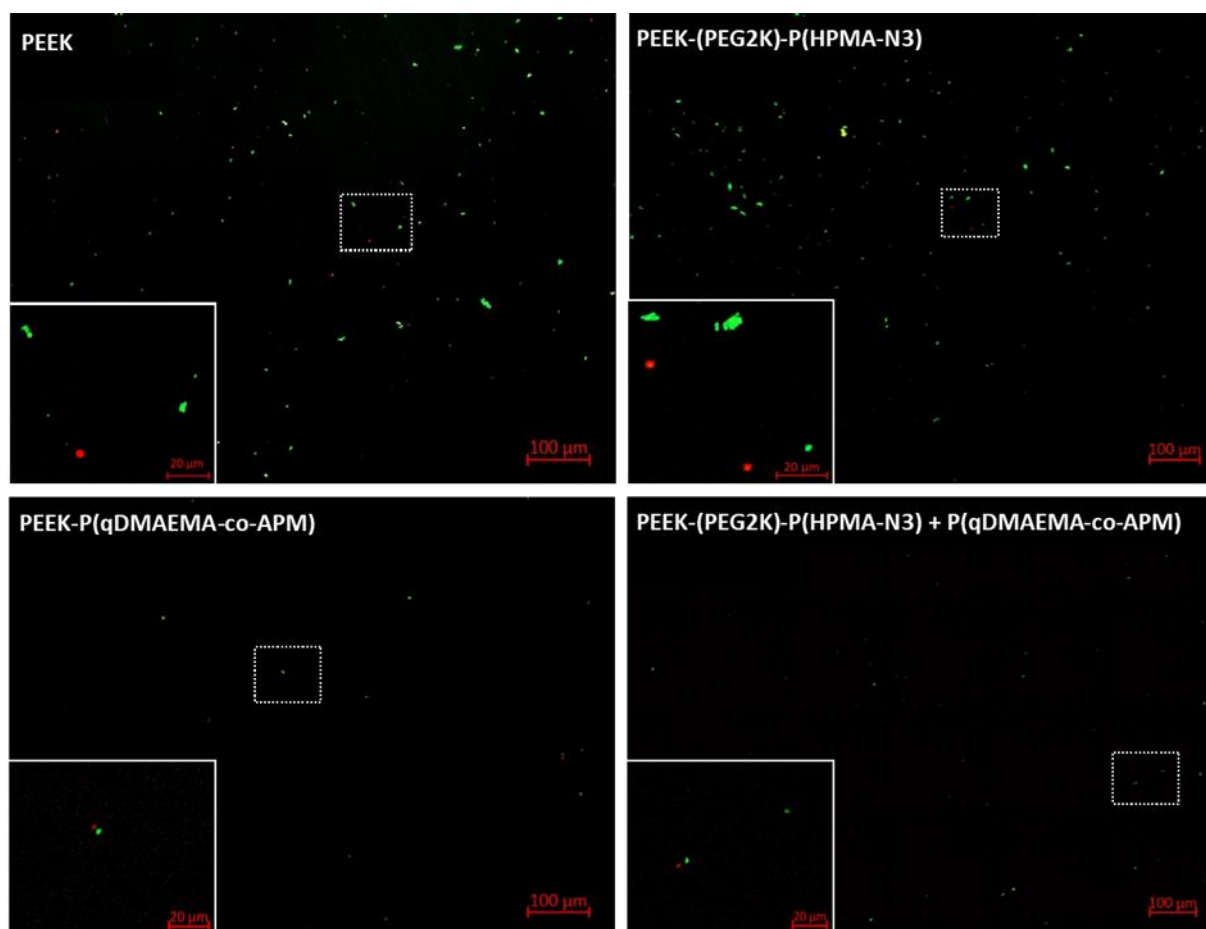


Figure S9. Live/Dead staining of bacteria cultured for 24 hours on PEEK surfaces.

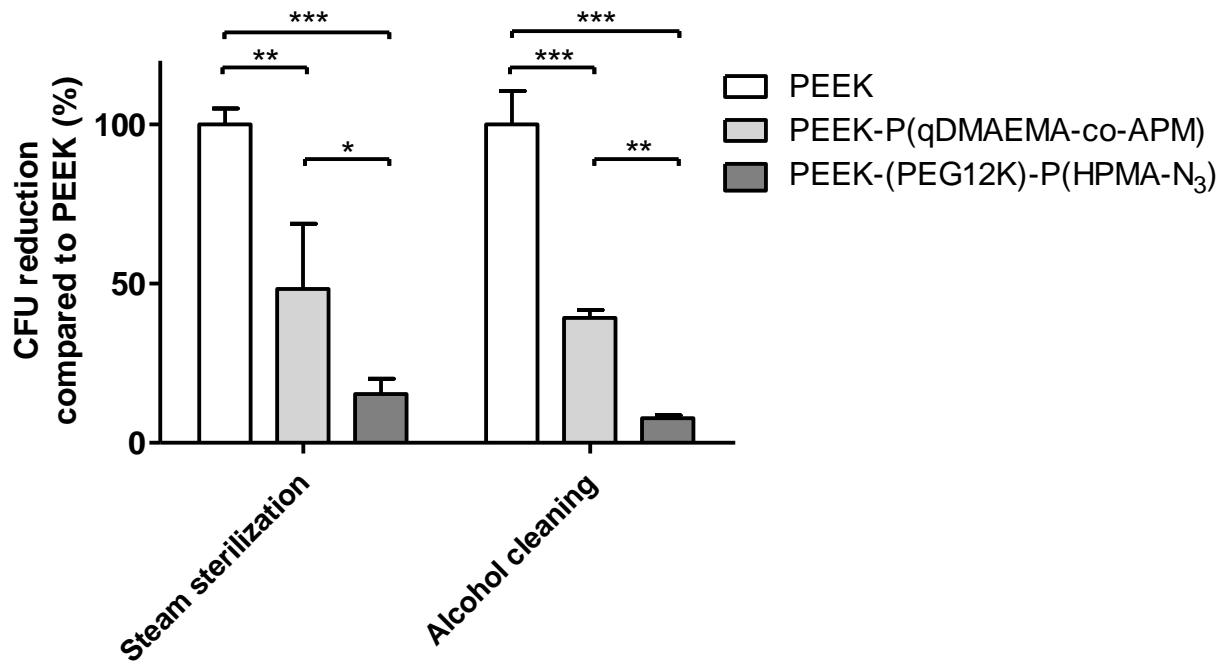


Figure S10. Direct assessment of cleaning and sterilization compatibility of the grafted polymers on the PEEK surface. Results within each method were normalized to the corresponding PEEK group. The data was subjected to 2-way ANOVA with Bonferroni post-test (* = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$).

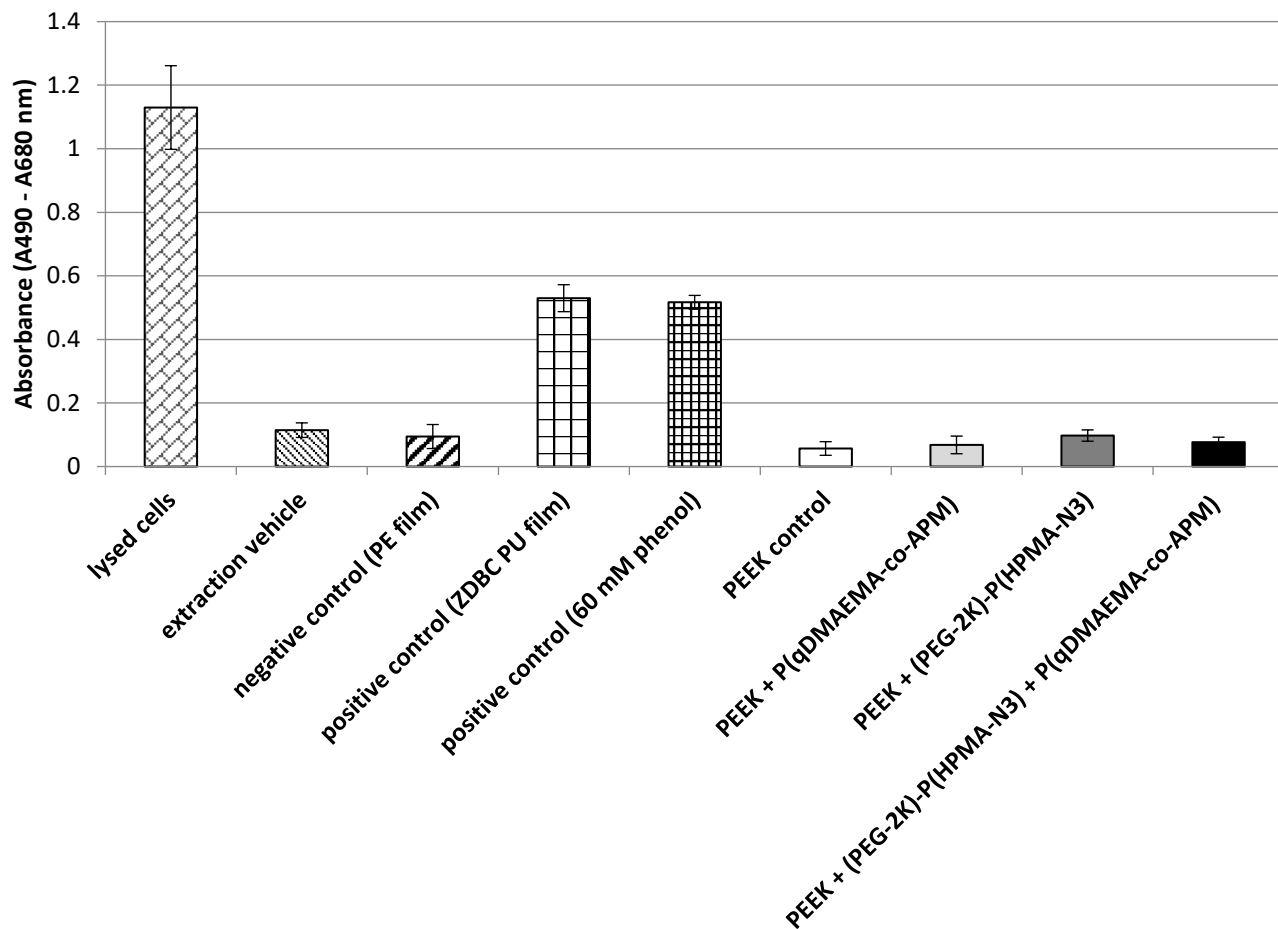


Figure S11. LDH cytotoxicity assay of fibroblasts grown in contact with extracts for 24 h. Data ($n \geq 3$) are expressed as mean \pm standard deviation.