

SUPPORTING INFORMATION

Multireactive Poly(2-oxazoline) Nanofibers through Electrospinning with Crosslinking on the Fly

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Materials

Poly(2-ethyl-2-oxazoline-co-2-(but-3-enyl)-2-oxazoline) (P(EtOx-co-ButEnOx): The ButenOx monomer was synthesized as first reported by Schlaad and coworkers.¹ The copolymer with an EtOx:ButenOx ratio of 190:10 and total degree of polymerization of 200 was reported following the microwave-assisted polymerization protocol as described in ref 38 in the main text. The resulting polymer has 6.7 mol% ButenOx as determined by ¹H NMR spectroscopy (Bruker Avance 300 spectrometer, 300 MHz, CDCl₃), a number average molar mass of 30700 g/mol and a dispersity of 1.14 as determined by size exclusion chromatography (Agilent 1260-series HPLC system equipped with a 1260 online degasser, a 1260 ISO-pump, a 1260 automatic liquid sampler (ALS), a thermostated column

compartment (TCC) at 50°C equipped with two PLgel 5 μ m mixed-D columns in series, a 1260 diode array detector (DAD) and a 1260 refractive index detector (RID). The used eluent was DMA containing 50mM of lithium chloride at an optimized flow rate of 0.593 ml/min). Pentaerythritol tetrakis(3-mercaptopropionate) (tetrathiol) and 2,2-dimethoxy-2-phenylacetophenone (DMPA) were purchased from Sigma Aldrich and Acros Organics, respectively. Tetramethylrhodamine-5-maleimide was purchased from Sigma. *N*-ethyl maleimide was purchased from Alfa Aesar. BODIPY-thiol was synthesized according to literature procedure.² Solvents were used as received.

Characterization

Scanning Electron Microscopy

Scanning electron microscopy (SEM) images of the nanofibers were taken by Philips XL30 ESEM-FEG/EDAX system at an operating voltage of 4 kV.

Fluorescence Microscopy

Fluorescence microscopy images of dry samples on nanofiber surfaces were recorded at room temperature using an LD-A-Plan 10 \times /0.30 objective in a Zeiss Observer Z1 fluorescence microscope (Zeiss Fluorescence Microscopy, Carl Zeiss Canada Ltd, Canada) connected to AxioCam MRc5 using a Zeiss Filter set 38 (Excitation BP 470/40, Emission BP 525/50) for imaging the functionalized nanofibers.

Goniometer

Static contact angle of a water droplet on nanofiber surfaces were measured under open-air condition using a goniometer (CAM 101 KSV instruments). Approximately 5 μ L of deionized water was deposited on the surface and images were taken by an integrated digital camera. The software CAM2008 was used for image processing to determine the contact angle. The contact angle value for each sample was independently measured at three different locations and average values are reported.

Electrospinning of crosslinked P(EtOx-co-ButEnOx) nanofibers

P(EtOx-co-ButEnOx) nanofibers were crosslinked using pentaerythritol tetrakis(3-mercaptopropionate) under UV in the presence of DMPA as the photoinitiator. P(EtOx-co-ButEnOx) (200 mg, 0.011 mmol), pentaerythritol tetrakis(3-mercaptopropionate) (48.86 mg, 0.1 mmol) and DMPA (5.12 mg, 0.02 mmol) were stirred in 1:1 DMF:THF solvent system (0.2 ml). M_n value of the polymer was used as 18039 g/mol, calculated with the correction factor $M_{n,PEtOx}=0.5876*M_{n,PMMA}$.³

The solution was placed in a 1 ml syringe with a needle and pumped with a flow rate of 0.01 ml/min. Nanofibers were electrospun under 13 kV voltage and 15 cm tip-to-collector distance on glass slides attached on a grounded aluminum collector. In-situ crosslinking was performed by UV during electrospinning.

Determination of sulfhydryl content

Free thiol group contents of the nanofibers were determined by Ellman's analysis.⁴ The sulfhydryl groups were quantitated using the extinction coefficient of TNB²⁻ (2-nitro-5-thiobenzoic acid) ion as the reference. Ellman's reagent solution was prepared by dissolving 4 mg Ellman's reagent (5,5-dithio-bis-(2-nitrobenzoic acid) in 1 ml of reaction medium composed of 0.1 M sodium phosphate, pH 8.0, containing 1 mM EDTA buffer. A nanofibrous sample (2.1 mg) was placed in a vial and 3 mL of buffer and Ellman's reagent solution were added on the sample. The resultant mixture was incubated at 37 °C for 2 h. The absorbance at 412 nm was measured to calculate the total sulfhydryl group content in the sample using the molar extinction coefficient of TNB²⁻ ($14,150 \text{ M}^{-1}\text{cm}^{-1}$). Using three different nanofiber samples thiol content was found as $4.65 \pm 0.45 \times 10^{-5} \text{ mmol/mg}$.

Fluorescent dye immobilization on nanofibers using thiol-ene reaction

A mixture of 10 μl of BODIPY-SH (1 mg/ml) and 1 μl of DMPA (2.4 mg/ml) solutions in THF was dropped on the glass slide covered with crosslinked nanofibers and the slide was placed under UV irradiation (365 nm at the distance of 10 cm) for 5 min. After the reaction, the sample was washed with excess THF to remove the unbound dye.

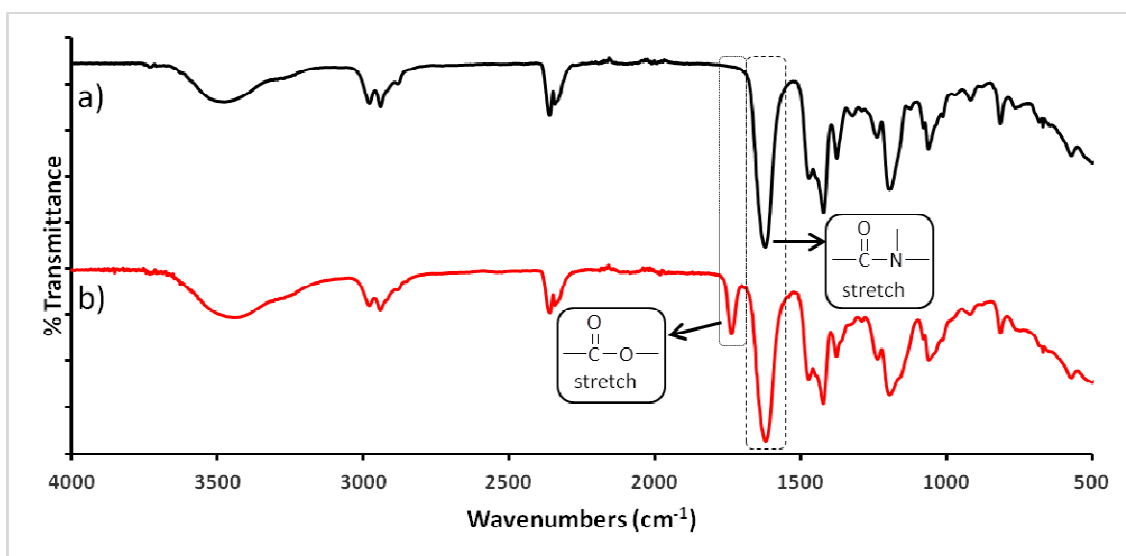
For the control experiments, a solution of *N*-ethylmaleimide (5 mg/ml) in THF was dropped on P(EtOx-co-ButEnOx)) nanofiber-coated glass slides prior to BODIPY-SH immobilization and the slides were washed with ample THF after 4h.

Spatially controlled functionalization of nanofibers using thiol-ene reaction

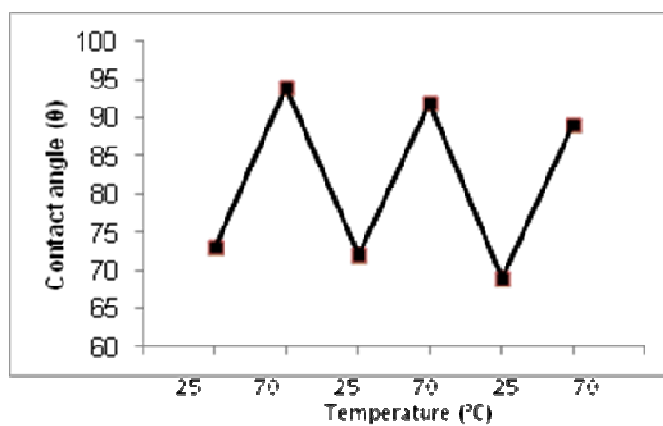
A mixture of 10 μl of BODIPY-SH (1 mg/ml) and 1 μl of DMPA (2.4 mg/ml) in THF were dropped on the glass slide covered with crosslinked nanofibers and a TEM grid as a UV mask was firmly placed onto the surface. The slide was placed under UV irradiation (365 nm at the distance of 10 cm) for 5 min. After the reaction, the sample was washed with excess THF to remove the unbound dye.

Fluorescent dye immobilization using Michael-addition

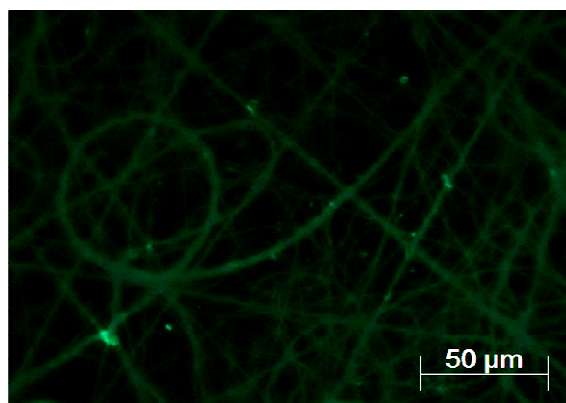
5 μl of tetramethylrhodamine-5-maleimide solution (0.35 mg/ml) in methanol was dropped on a glass slide covered with crosslinked nanofibers and was left for 4 h in the dark. The sample was washed with ample methanol in order to remove the excess dye.



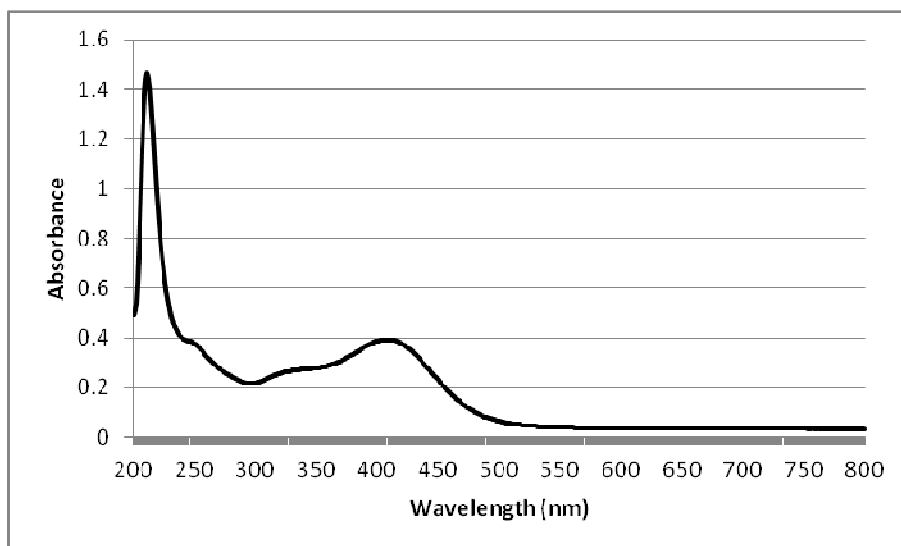
S-1. FT-IR spectra of a) non-crosslinked (in black) and b) crosslinked P(EtOx-co-ButEnOx) nanofibers.



S-2. Graph showing the thermoresponsive behavior of the nanofibers probed with change in water contact angle with temperature.



S-3. Fluorescence microscopy images of BODIPY-SH treated nanofibers without UV exposure before *N*-ethylmaleimide conjugation as a control experiment.



S-4. Uv spectrum of nanofibers after Ellman's analysis showing the absorbance of TNB^{2-} at 412 nm

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