

## Supporting Information

# **Bone-Adhesive Barrier Membranes Based on Alendronate-Functionalized Poly(2-oxazoline)s**

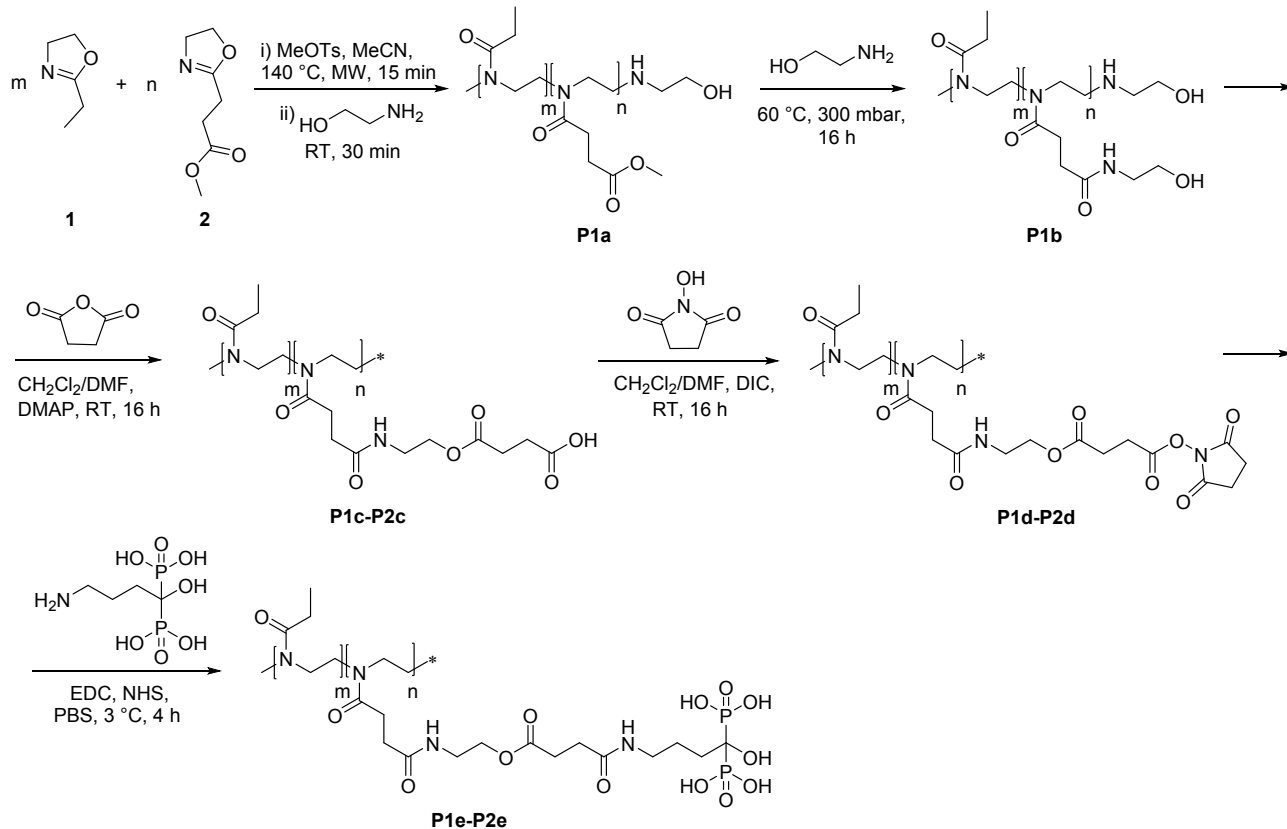
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### Scheme 1. Synthetic Route of Alendronate-Functionalized Poly(2-oxazoline)s.



The end groups depicted as \* are similar to the functionalized side chains shown in each reaction step.

### S1. Procedures for the Synthesis of Alendronate-Functionalized Poly(2-oxazoline)s.

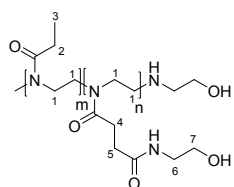
#### a) Polymerization: Synthesis of Methyl Ester-Functionalized Polymer

**P(EtOx<sub>70</sub>-MestOx<sub>30</sub>) P1a.** Methyl-*p*-toluenesulfonate (1 equiv, 0.80 mL, 5.27 mmol), 2-ethyl-2-oxazoline **1** (EtOx, 70 equiv, 37.26 mL, 368.94 mmol), 2-methoxycarbonylethyl-2-oxazoline **2** (MestOx, 30 equiv, 21.80 mL, 158.12

mmol), and dry MeCN (73 mL, 4 M) were mixed under an argon atmosphere in the desired ratios in microwave vials. The polymerization was carried out under microwave irradiation at 140 °C for

15 min following by CROP. After polymerization, the reaction was terminated by the addition of 2-ethanolamine (10 equiv, 3.18 mL, 52.71 mmol) while stirring for 30 min at room temperature. Then, the solvent was removed in vacuo to afford P(EtOx-*r*-MestOx) **P1a** (60.56 g, 5.16 mmol) as statistical copolymer with near-random monomer distribution in the desired ratio. <sup>1</sup>H NMR [400 MHz, δ (ppm), CDCl<sub>3</sub>]: 3.65 (br, 3 H, 5-CH<sub>3</sub>), 3.70–3.35 (br, 8 H, 1-CH<sub>2</sub>), 2.70–2.50 (br, 4 H, 4-CH<sub>2</sub>), 2.50–2.20 (br, 2 H, 2-CH<sub>2</sub>), 1.20–1.00 (br, 3 H, 3-CH<sub>3</sub>). Experimentally determined comonomer ratio: *m/n* 70:30. SEC: *M<sub>n</sub>* 10.6 kDa, *D* 1.10. MALDI-TOF MS: *M<sub>n</sub>* 10.6 kDa. Yield: 98%.

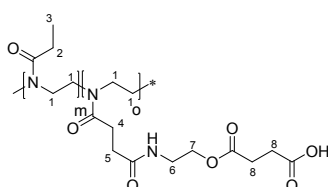
**b) Amidation Reaction: Synthesis of Hydroxyl-Functionalized Polymer P(EtOx<sub>70</sub>-OH<sub>30</sub>) P1b.**



The MestOx-functionalized copolymer **P1a** (1 equiv, 60.05 g, 5.11 mmol) was modified by direct amidation with 2-ethanolamine (3.5 equiv, 32.41 mL, 537.02 mmol) at 60 °C under reduced pressure (300 mbar) for 16 h. Then, the

crude mixture was purified by three consecutive precipitations in a mixture of acetone/Et<sub>2</sub>O, 3:1, and subsequent re-dissolution in CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 8:2, followed by ion-exchange chromatography in MeOH. Finally, the solvent was removed in vacuo to afford P(EtOx-*r*-OH) **P1b** (49.69 g, 3.94 mmol) as statistical copolymer with near-random monomer distribution in the desired ratio. <sup>1</sup>H NMR [400 MHz, δ (ppm), D<sub>2</sub>O]: 3.65 (br, 2 H, 7-CH<sub>2</sub>), 3.75–3.45 (br, 8 H, 1-CH<sub>2</sub>), 3.35–3.25 (br, 2 H, 6-CH<sub>2</sub>), 2.75–2.60 (br, 2 H, 5-CH<sub>2</sub>), 2.60–2.50 (br, 2 H, 4-CH<sub>2</sub>), 2.45–2.25 (br, 2 H, 2-CH<sub>2</sub>), 1.15–1.00 (br, 3 H, 3-CH<sub>3</sub>). Experimentally determined comonomer ratio: *m/n* 70:30. MALDI-TOF MS: *M<sub>n</sub>* 11.1 kDa. Yield: 77%.

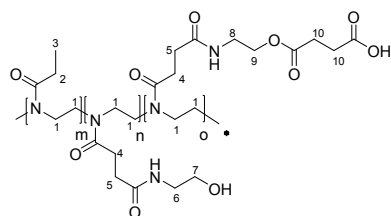
**c) Succinic Anhydride Coupling: Synthesis of Carboxylic Acid-Functionalized Polymer P(EtOx<sub>70</sub>-COOH<sub>30</sub>) P1c.**



The hydroxyl side-functionalized polymer **P1b** (1 equiv, 24.00 g, 1.90 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub>/DMF,

(9:1, 41 mL, 2 M) under an argon atmosphere. Succinic anhydride (1.1 equiv, 6.85 g, 68.49 mmol) and 4-dimethylamino pyridine (DMAP, 0.2 equiv, 1.40 g, 11.42 mmol) were added to the mixture and it was stirred at room temperature for 16 h. The crude mixture was purified by three consecutive precipitations in acetone and subsequent re-dissolution in CH<sub>2</sub>Cl<sub>2</sub>/MeOH, 8:2, followed by ion-exchange chromatography in MeOH. Finally, the solvent was removed in vacuo to afford P(EtOx-*r*-COOH) **P1c** (24.41 g, 1.57 mmol) as statistical copolymer with near-random monomer distribution in the desired ratio. <sup>1</sup>H NMR [400 MHz, δ (ppm), D<sub>2</sub>O]: 4.25–4.15 (br, 2 H, 7-CH<sub>2</sub>), 3.75–3.40 (br, 10 H, 1-CH<sub>2</sub> + 6-CH<sub>2</sub>), 2.75–2.60 (br, 6 H, 5-CH<sub>2</sub> + 8-CH<sub>2</sub>), 2.60–2.45 (br, 2 H, 4-CH<sub>2</sub>), 2.45–2.25 (br, 2 H, 2-CH<sub>2</sub>), 1.15–1.00 (br, 3 H, 3-CH<sub>3</sub>). Experimentally determined comonomer ratio: *m/o* 70:30. MALDI-TOF MS: *M<sub>n</sub>* 13.5 kDa. Yield: 82%.

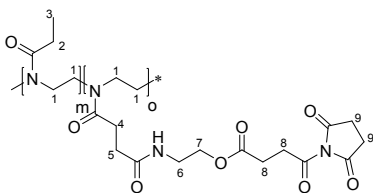
#### Synthesis of Carboxylic Acid-Functionalized Polymer



**P(EtOx<sub>70</sub>-OH<sub>10</sub>-COOH<sub>20</sub>) P2c.** According to the procedure described above, **P2c** was synthesized by partially conversion of hydroxyl groups to carboxylic acid moieties by using fewer

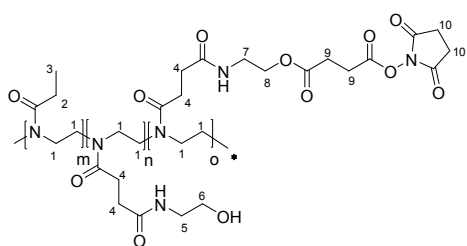
amounts of succinic anhydride. The reaction of a solution of **P2b** (25.01 g, 1.98 mmol), DMAP (0.97 g, 7.93 mmol), and succinic anhydride (4.76 g, 47.56 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/DMF (9:1, 29 mL, 2 M) afforded the desired polymer P(EtOx-*r*-OH-*r*-COOH) **P2c** (26.05 g, 1.78 mmol). <sup>1</sup>H NMR [400 MHz, δ (ppm), D<sub>2</sub>O]: 4.25–4.15 (br, 2 H, 9-CH<sub>2</sub>), 3.75–3.40 (br, 16 H, 1-CH<sub>2</sub> + 7-CH<sub>2</sub> + 8-CH<sub>2</sub>), 3.35–3.20 (br, 2 H, 6-CH<sub>2</sub>), 2.75–2.60 (br, 8 H, 5-CH<sub>2</sub> + 10-CH<sub>2</sub>), 2.60–2.45 (br, 4 H, 4-CH<sub>2</sub>), 2.45–2.25 (br, 2 H, 2-CH<sub>2</sub>), 1.15–1.00 (br, 3 H, 3-CH<sub>3</sub>). Experimentally determined comonomer ratio: *m/n/o* 70:11:19. MALDI-TOF MS: *M<sub>n</sub>* 12.7 kDa. Yield: 90%.

#### d) Carbodiimide Reaction: Synthesis of *N*-Hydroxysuccinimide-Functionalized Polymer P(EtOx<sub>70</sub>-



**NHS<sub>30</sub>) P1d.** The carboxyl-functionalized polymer **P1c** was subsequently modified into reactive ester by carbodiimide coupling with NHS, to facilitate the next amidation reaction. The functionalized polymer **P1c** (1 equiv, 24.02 g, 3.91 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub>/DMF (95:5, 530 mL, 0.2 M), *N*-hydroxysuccinimide (NHS, 1.1 equiv, 14.85 g, 129.00 mmol) and *N,N'*-diisopropylcarbodiimide (DIC, 1.2 equiv, 21.79 mL, 140.72 mmol) were added as coupling agents, and the mixture was stirred under an argon atmosphere at room temperature for 16 h. The polymer was purified by two precipitations in acetone/Et<sub>2</sub>O, 1:1, followed by another precipitation in Et<sub>2</sub>O and subsequent re-dissolution in CH<sub>2</sub>Cl<sub>2</sub>. Finally, the solvent was removed in vacuo to afford P(EtOx-*r*-NHS) **P1d** (23.78 g, 1.28 mmol) as statistical copolymer with near-random monomer distribution in the desired ratio. <sup>1</sup>H NMR [400 MHz, δ (ppm), DMSO-d<sub>6</sub>]: 4.00–3.95 (br, 2 H, 7-CH<sub>2</sub>), 3.50–3.20 (br, 10 H, 1-CH<sub>2</sub> + 6-CH<sub>2</sub>), 3.00–2.90 (br, 2 H, 8-CH<sub>2</sub>), 2.85–2.75 (br, 4 H, 9-CH<sub>2</sub>), 2.75–2.65 (br, 2 H, 8-CH<sub>2</sub>), 2.60–2.40 (br, 2 H, 5-CH<sub>2</sub>), 2.40–2.20 (br, 4 H, 2-CH<sub>2</sub> + 4-CH<sub>2</sub>), 1.00–0.90 (br, 3 H, 3-CH<sub>3</sub>). Experimentally determined comonomer ratio: *m/o* 70:30. SEC: *M*<sub>n</sub> 15.6 kDa, *D* 1.23. MALDI-TOF MS: *M*<sub>n</sub> 16.9 kDa. Yield: 84%.

#### Synthesis of *N*-Hydroxysuccinimide-Functionalized Polymer P(EtOx<sub>70</sub>-OH<sub>10</sub>-NHS<sub>20</sub>) **P2d**.



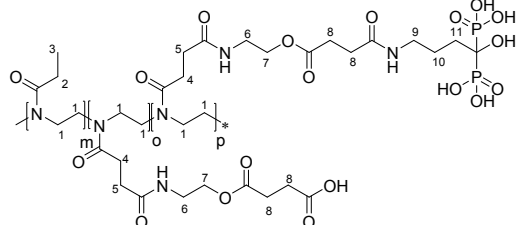
According to the procedure described above, the reaction of a solution of **P2c** (25.50 g, 1.76 mmol), NHS (4.23 g, 36.73 mmol) and DIC (6.20 mL, 40.07 mmol) in CH<sub>2</sub>Cl<sub>2</sub>/DMF (95:5, 387 mL, 0.2 M) afforded the desired polymer P(EtOx-

*r*-OH-*r*-NHS) **P2d** (24.61 g, 1.51 mmol). <sup>1</sup>H NMR [400 MHz, δ (ppm), DMSO-d<sub>6</sub>]: 4.05–3.90 (br, 2 H, 8-CH<sub>2</sub>), 3.60–3.15 (br, 16 H, 1-CH<sub>2</sub> + 6-CH<sub>2</sub> + 7-CH<sub>2</sub>), 3.15–3.00 (br, 2 H, 5-CH<sub>2</sub>), 2.95–2.85 (br, 2 H, 9-CH<sub>2</sub>), 2.85–2.70 (br, 4 H, 10-CH<sub>2</sub>), 2.70–2.60 (br, 2 H, 9-CH<sub>2</sub>), 2.60–2.40 (br, 8 H, 4-CH<sub>2</sub>), 2.35–2.15 (br, 2 H, 2-CH<sub>2</sub>), 1.00–0.85 (br, 3 H, 3-CH<sub>3</sub>). Experimentally determined

comonomer ratio: *m/n/o* 70:11:19. SEC:  $M_n$  14.7 kDa,  $D$  1.25. MALDI-TOF MS:  $M_n$  14.4 kDa.

**Yield:** 86%.

### e) Amidation Reaction: Synthesis of Alendronate-Functionalized Polymer P(EtOx<sub>70</sub>-Ale<sub>30</sub>)

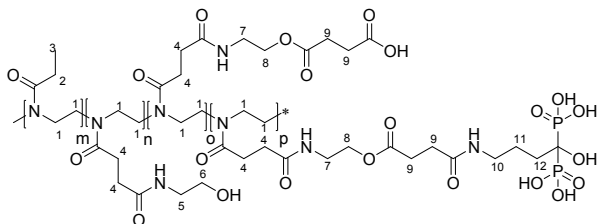


**P1e.** Alendronate moieties were incorporated in the polymer side chain by an amidation reaction. The NHS-activated copolymer **P1d** (1 equiv, 12.00 g, 0.65 mmol) was slowly added into a solution containing sodium

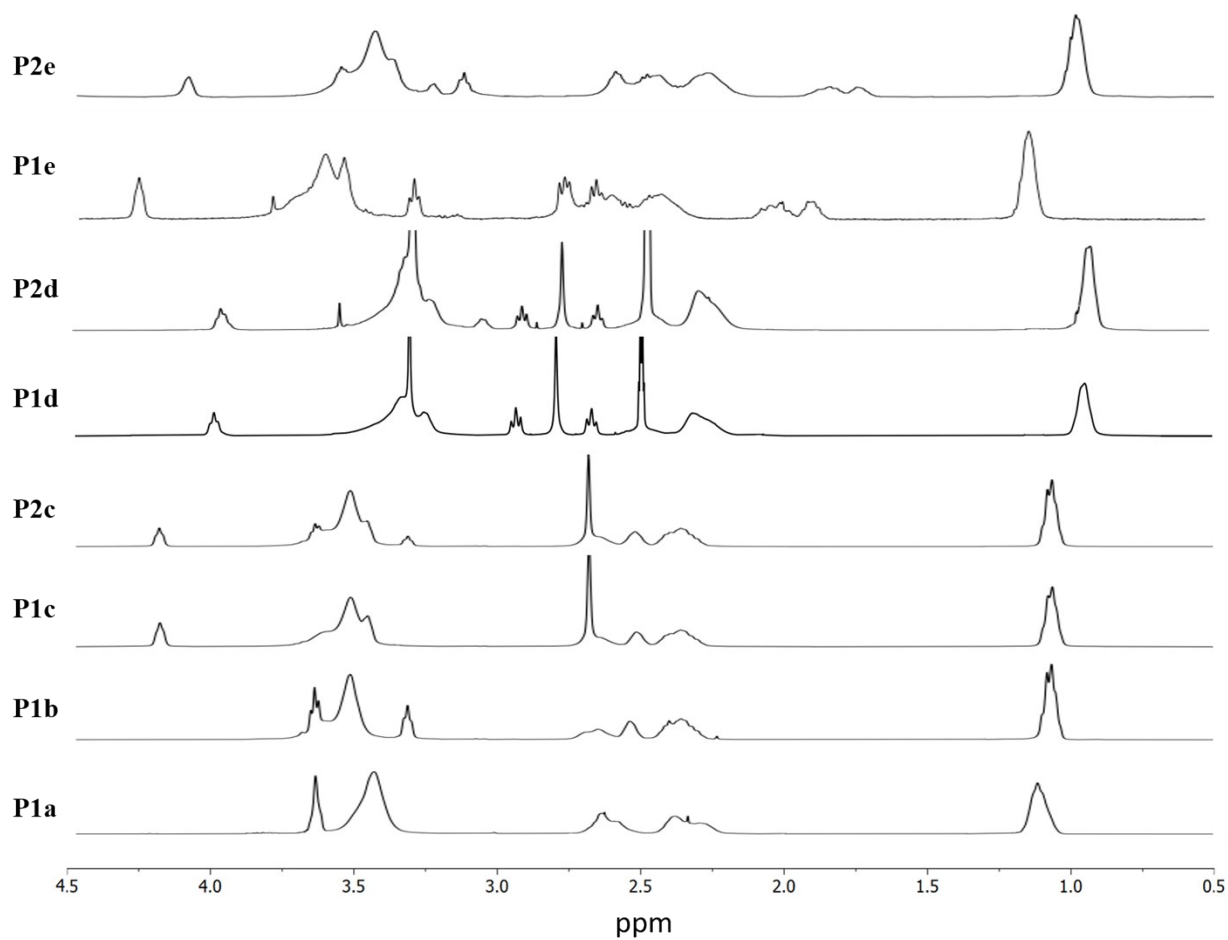
alendronate trihydrate (Ale, 1.5 equiv, 9.48 g, 29.17 mmol), NHS (1 equiv, 2.24 g, 19.44 mmol), and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC, 1 equiv, 3.73 g, 19.44 mmol) in PBS (137 mL, 0.5 M). NHS and EDC were added to maximize the conversion from NHS ester to alendronate-functionalized polymers, and thus minimize premature hydrolysis of NHS esters. The reaction mixtures were stirred at 3 °C for 4 h with the pH adjusted constantly to 7.4–8.0 using NaOH (0.5 M). Afterward, the crude mixture was dialyzed using Spectra/Por® 3 membranes (3.5 kDa cutoff) for 4 h. Then, it was lyophilized, dissolved in MeOH and purified using a soxhlet extractor. Finally, the solvent was removed in vacuo to afford P(EtOx-*r*-Ale) **P1e** (7.38 g, 0.33 mmol) as statistical copolymer with near-random monomer distribution in the desired ratio. <sup>1</sup>H NMR [400 MHz, δ (ppm), D<sub>2</sub>O]: 4.30–4.20 (br, 4 H, 7-CH<sub>2</sub>), 3.90–3.40 (br, 16 H, 1-CH<sub>2</sub> + 6-CH<sub>2</sub>), 3.35–3.25 (br, 2 H, 9-CH<sub>2</sub>), 2.85–2.30 (br, 18 H, 2-CH<sub>2</sub> + 4-CH<sub>2</sub> + 5-CH<sub>2</sub> + 8-CH<sub>2</sub>), 2.15–1.95 (br, 2 H, 10-CH<sub>2</sub>), 1.95–1.80 (br, 2 H, 11-CH<sub>2</sub>), 1.25–1.00 (br, 3 H, 3-CH<sub>3</sub>). <sup>31</sup>P NMR [400 MHz, δ (ppm), D<sub>2</sub>O]: 18.20. Experimentally determined comonomer ratio: *m/o/p* 70:3:27. MALDI-TOF MS:  $M_n$  19.6 kDa. **Yield:** 51%.

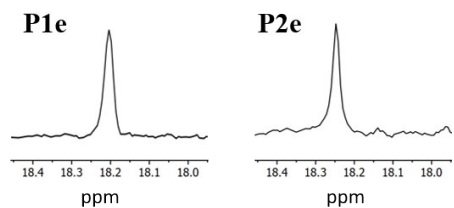
### Synthesis of Alendronate-Functionalized

Polymer P(EtOx<sub>70</sub>-OH<sub>10</sub>-Ale<sub>20</sub>) **P2e**. According

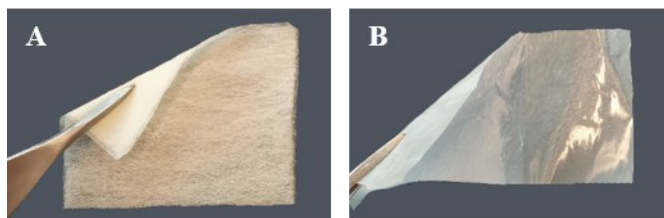


to the procedure described above, the reaction of a solution of **P2d** (12.00 g, 0.73 mmol), Ale (6.80 g, 20.92 mmol), NHS (1.60 g, 13.94 mmol) and EDC (2.67 g, 13.94 mmol) in PBS (99 mL, 0.5 M) afforded the desired polymer **P2e** (8.97 g, 0.47 mmol). **<sup>1</sup>H NMR** [400 MHz,  $\delta$  (ppm), D<sub>2</sub>O]: 4.15–4.00 (br, 4 H, 8-CH<sub>2</sub>), 3.70–3.30 (br, 22 H, 1-CH<sub>2</sub> + 6-CH<sub>2</sub> + 7-CH<sub>2</sub>), 3.30–3.20 (br, 2 H, 5-CH<sub>2</sub>), 3.20–3.10 (br, 2 H, 10-CH<sub>2</sub>), 2.70–2.15 (br, 22 H, 2-CH<sub>2</sub> + 4-CH<sub>2</sub> + 9-CH<sub>2</sub>), 1.95–1.65 (br, 4 H, 11-CH<sub>2</sub> + 12-CH<sub>2</sub>), 1.10–0.90 (br, 3 H, 3-CH<sub>3</sub>). **<sup>31</sup>P NMR** [400 MHz,  $\delta$  (ppm), D<sub>2</sub>O]: 18.25. Experimentally determined comonomer ratio: *m/n/o/p* 70:11:1:18. **MALDI-TOF MS**: *M<sub>n</sub>* 14.9 kDa. **Yield**: 64%.

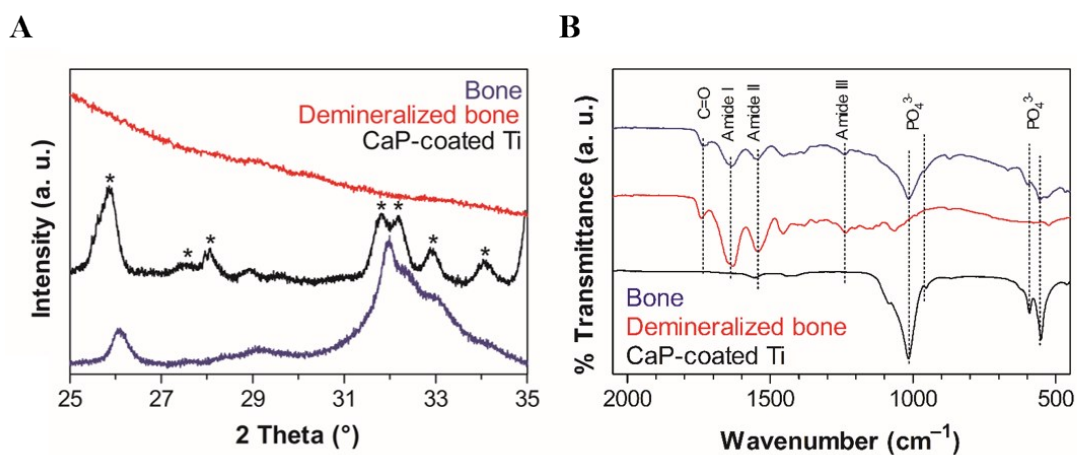




**Figure S1.**  $^1\text{H}$  NMR (top) and  $^{31}\text{P}$  NMR (bottom) spectra of synthesized polymers.

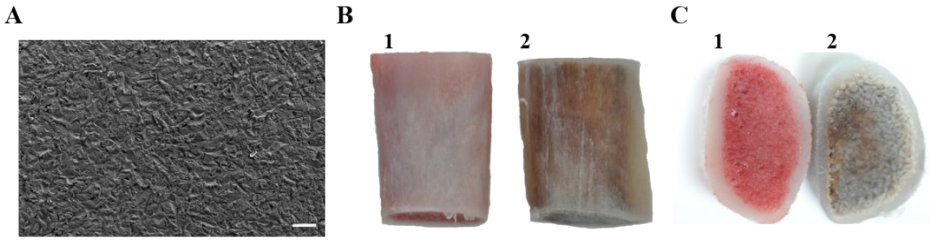


**Figure S2.** A) Blank (carrier with backing) and B) backing layer.

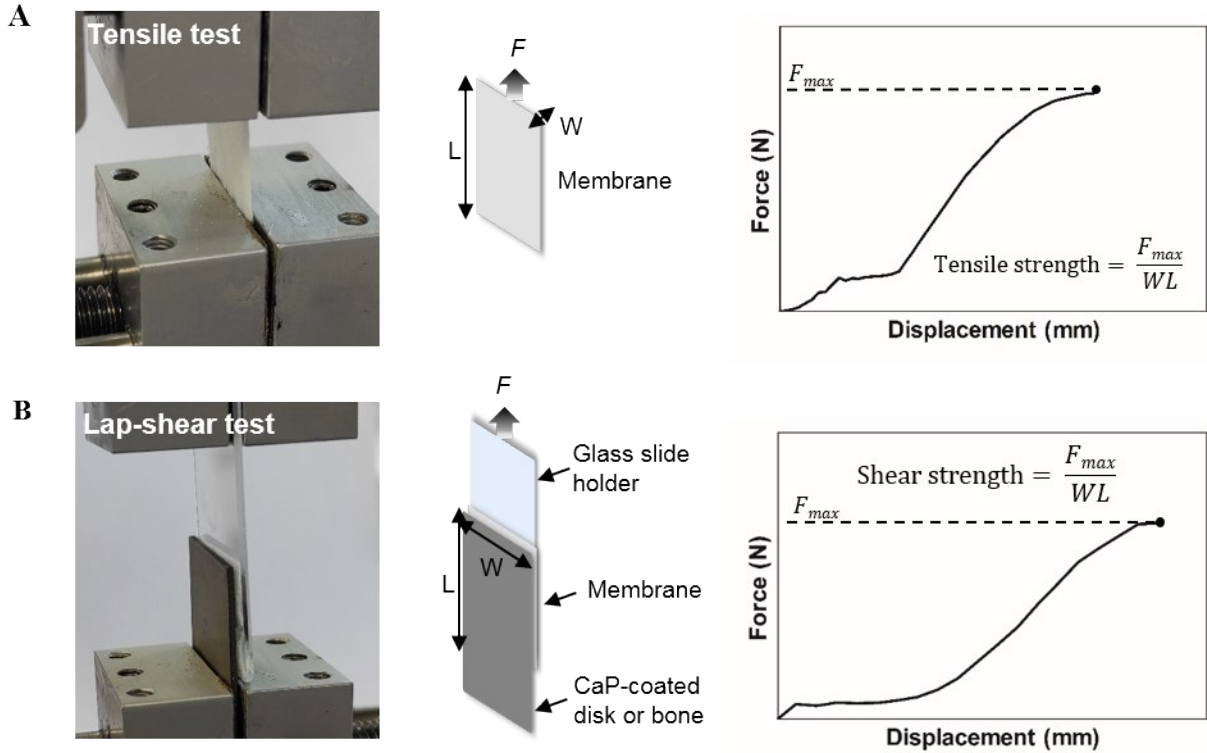


**Figure S3.** A) Thin-film X-ray diffractogram of CaP-coated disks (peaks characteristic of nanocrystalline calcium apatite are indicated with an asterisk), bone, and demineralized bone specimens. B) Fourier transform infrared spectrum of CaP-coated disks, bone, and demineralized bone specimens.

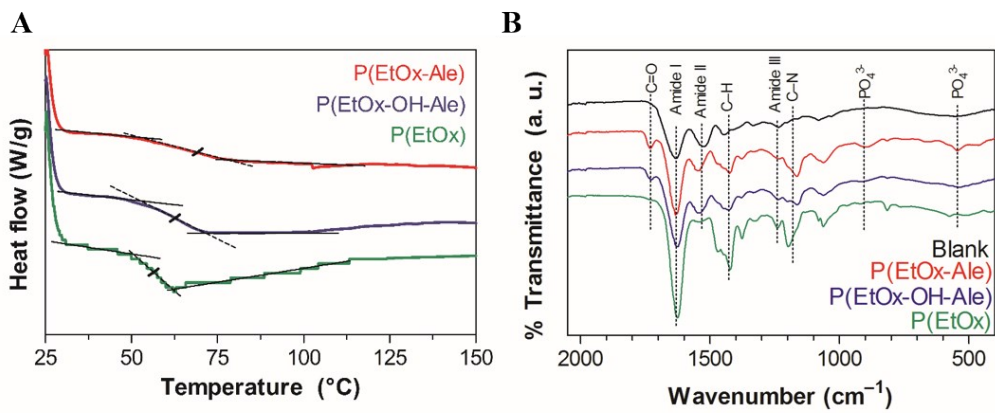




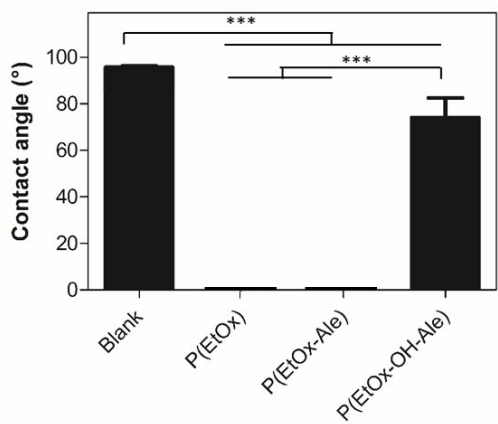
**Figure S4.** A) Scanning electron micrographs of CaP-coated disks (scale bar correspond to 100  $\mu\text{m}$ ). B) Longitudinal and C) cross-sectional views of bone specimens: 1) before and 2) after demineralization.



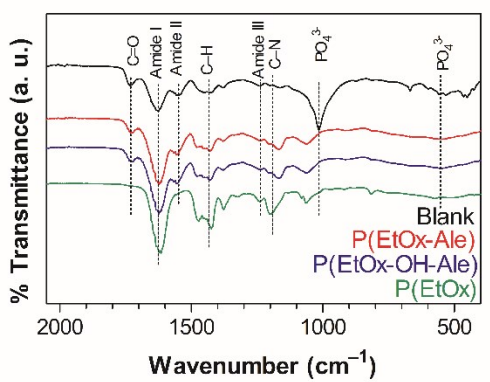
**Figure S5.** Experimental setups for A) tensile testing and B) lap-shear adhesion testing.



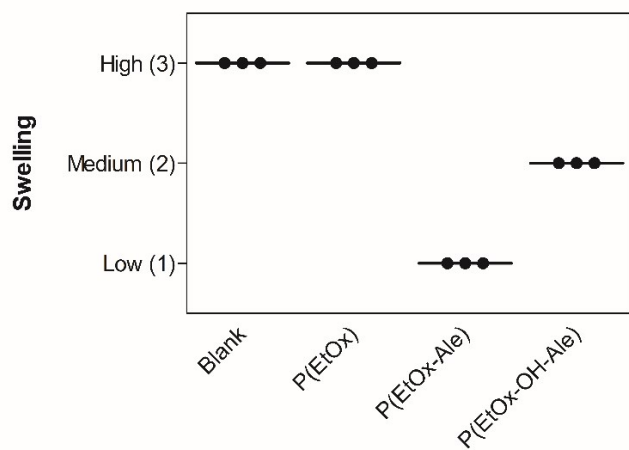
**Figure S6.** A) Thermograms of POx polymers and B) FTIR spectra of the various experimental groups.



**Figure S7.** Water contact angle of the membranes after 1 sec of contact.



**Figure S8.** FTIR spectra of bone specimens after lap-shear testing of various membranes.



**Figure S9.** Swelling of membranes under wet conditions.

**Table S1.** Physical Characterization of the Tested Membranes ( $15 \times 25 \text{ mm}^2$ ).

Prototype	Thickness (mm)	Density ( $\text{g/cm}^3$ )	Weight ratio polymer/carrier/backing
Carrier	$0.28 \pm 0.02$	$0.31 \pm 0.08$	0:100:0
Backing	$0.05 \pm 0.00$	$1.70 \pm 0.08$	0:0:100
Blank	$0.28 \pm 0.02$	$0.39 \pm 0.03$	0:48:52
P(EtOx)	$0.31 \pm 0.02$	$0.58 \pm 0.04$	42:28:30
P(EtOx-Ale)	$0.25 \pm 0.03$	$0.55 \pm 0.07$	42:28:30
P(EtOx-OH-Ale)	$0.31 \pm 0.04$	$0.50 \pm 0.04$	42:28:30