Electronic Supplementary Information (ESI)

Designing a multifaceted bio-interface nanofiber tissue-engineered tubular scaffold graft to promote neo-vascularization for urethral regeneration

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Materials characterizations

1.1 Thermal analysis. Differential scanning calorimetry (DSC) and thermogravimetry analysis (TGA) were performed on TA Instruments Q100 and Q50 respectively under nitrogen atmospheres. The DSC analysis was as following: a sample of 2.5 mg in an aluminum pan was cooled from room temperature to -60 °C by an auto cool accessory, the pan was heated from -60 °C to 180 °C at a 10 °C /min rate, isothermally maintained at 180 °C for 3 min, quenched to -60 °C, and reheated from -60 °C to 180 °C at 10 °C /min under a nitrogen flow rate of 50 mL/min. Data were collected during the second heating run. The glass-transition temperature (Tg) was taken as the midpoint of the heat capacity change. Melting point (Tm) was taken as the summit of melting peak and melting enthalpy (DHm) was calculated from the area of the endothermic peak.
Samples for TGA were heated at a heating rate of 10 °C/min from room temperature to 500.

2.2 The cyclic tensile tests. Elongational experiments were conducted with an Anton Paar MCR 702 rotational rheometer (Graz, Austria) at room temperature (20°C) using a uniaxial extensional fixture (UXF), in which the sample is wound up on 2 counter-rotating drums, allowing for homogeneous high deformations. The sample length is given by the geometry (ca. 15 mm plus several more mm for clamping). The sample cross-section was between 3 and 8 mm in width and 0.1 and 2 mm in thickness, which was given by the thickness of the electrospun fibers or the wall thickness of the urethras and the width of the samples was adjusted accordingly to obtain a suitable force for the experiments that would be performed. The samples were clamped to the geometry and additional fixed by superglue to avoid slipping in the beginning of the experiments.

For all experiments a Hencky-strain\(^{1-2}\) rate \(\dot{\varepsilon} = 0.1 \, \text{s}^{-1}\) was used, which is an exponentially increasing stretching speed. The stress was evaluated in terms of physical and not engineering stress, i.e. the stress was determined based on the cross-section at the current deformation and not on the cross-section in the beginning of the experiment. The sample was stretched to a Hencky strain \(\varepsilon_H\), which is below the strain at break and
then brought back to $\varepsilon_H = 0$. This procedure was repeated 9 times. The Young’s modulus was determined from the average and standard deviation of 20-50 secants at small deformation ($\varepsilon_H = 0.015-0.12$) for each sample individually and then averaged. Typically, this leads to standard deviations below 3%.

![Chemical structure](image)

**Fig. S1** $^1$H NMR spectrum of PU-alt in CDCl$_3$. 

![NMR spectrum](image)
**Fig. S2** Characteristics of the PU-alt nanofiber scaffolds. (A) Scanning electron microscope (SEM) and polarized light microscope (PLM) images of PCL, E4-alt-C20, E10-alt-C20, and E20-alt-C20 nanofiber scaffolds with different hydrophilic/hydrophobic surfaces. The insets are water contact angle images of corresponding scaffolds. (B) After 48 h coculturing, morphologies of rabbit smooth muscle cells (SMCs) and epithelial cells (ECs) on different substrates, respectively, observed by SEM. Scale bars:
15 μm. (C) Average areas of SMCs and ECs after culture on different substrates for 72 h. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ ($n = 6/\text{mm}^2$ area). (D) The stress-strain curve of PCL, E4-$alt$-C20, E10-$alt$-C20, and E20-$alt$-C20 nanofiber scaffolds under wet conditions.

![XRD pattern of PCL, E4-$alt$-C20, E10-$alt$-C20, and E20-$alt$-C20 nanofiber scaffolds surface.](image)

Fig. S3 The XRD pattern of PCL, E4-$alt$-C20, E10-$alt$-C20, and E20-$alt$-C20 nanofiber scaffolds surface.
Fig. S4 In vitro cell phenotypic expression and matrix synthesis.

Immunocytochemical analysis of the protein expression of (A, C, E, G) α-SMA (green staining) of SMCs, and (B, D, F, H) AE1/AE3 (green staining) of
ECs as well as their respective elastin (red staining) on different substrates at 72 hours of cultivation, respectively. Scale bars, 180 μm.

Fig. S5 Morphology observation of tissue-engineered autologous urethra scaffolds prior to implantation. Fluorescent staining of the cross-section of tissue-engineered autologous PU-alt (A) and PCL (E) at low magnification. (C) Macroscopic image of the auto-urethral tissue. (B), (D) and (F) higher magnification of yellow box from figure (A), (C), and (E), respectively. Scale bars, 100 μm.
Fig. S6 *In vivo* scaffold replacement in New Zealand rabbit urethras. (A) Urethrotomy and exposure of urethral lumen, (B-C) an end-to-end anastomosis procedure with the tissue-engineered autologous PU-alt tubular scaffold graft (2.2 cm length) implantation, (D) Auto urethral tissue graft was implanted to the defect space. Urethras photographs of (E) tissue-engineered autologous PU-alt, (F) autograft, and (G) tissue-engineered autologous PCL scaffolds at 14 weeks post-operation; (H) Urethras photograph of rabbit in blank group after 14 weeks.
Fig. S7 Histological analysis of the mid-section of regenerated urethras at 90 days postoperatively: (A-D) Hematoxylin and eosin staining, (E-H) Immunofluorescence staining. (A, E) Tissue-engineered autologous PU-alt; (B, F) Autograft; (C, G) Tissue-engineered autologous PCL scaffold; (D, H) Blank control group. Scale bars, 1 mm. Red arrows indicate urethral stricture site.
Fig. S8 *In vivo* cell phenotype expression analysis. Confocal laser scanning microscopy (CLSM) images of the mid-section of regenerated urethras after transplantation of tissue-engineered autologous PU-*alt* scaffold graft at pre-determined time points postoperatively. Scale bars, 1 mm. Red arrows indicate vascular endothelial cell within the PU-*alt* scaffold; white arrows indicate degraded PU-*alt* fiber within the PU-*alt* scaffolds.
**Fig. S9** *In vivo* lumen epithelialization process. (A, B) Masson’s trichrome staining at 40 × of the tissue engineered PU-*alt* urethral scaffold at the predetermined time points after implantation. (C, D) Hematoxylin and eosin staining of the same cross-section for blood vessels and collagen. Black arrows indicate the ECM layer that secreted by already seeded ECs in the inner layer of PU-*alt* scaffold. red arrows indicate lined with neo-vessels in the PU-*alt* scaffold. Scale bars, 50 μm.
**Fig. S10** Histological analysis of regenerated urethra after various tissue-engineered urethra scaffolds transplantation into the urethral defect space. (A-E) Sirius red staining at ×10 of the mid-section of the regenerated urethras after transplantation of tissue-engineered autologous PU-alt scaffold at pre-determined time points postoperatively. Scale bars, 1 mm. (A1-E1) The insets indicate the columnar epithelial tissue (yellow) and collagen (orange yellow) in the inner wall of the regenerated urethra. (A2-E2) Hematoxylin & eosin
staining the nuclei (purple blue) and the components in cytoplasm and extracellular matrix (red) of columnar epithelial tissue in the inner wall of the regenerated urethra. (A3-E3) Masson’s trichrome staining of the same cross-section stained for the columnar epithelial tissue, surround by blood vessels (red) and collagen (blue) at × 63. Scale bars: (A1-E1), (A2-E2),(A3-E3) 50 μm.
Fig.S11 DSC thermograms (2nd heating run) of PU-alt copolymers and their pre-polymers (10 °C/min).

Fig.S12 TGA thermograms of PU-ran copolymers and their prepolymer.
Fig. S13. The cyclic tensile tests of regenerated urethras and PU-alt (E10-alt-C20) scaffolds. Cyclic test of (A) autograft, (B) PU-alt regenerated urethra (C) PCL-regenerated urethra and (D) E10-alt-C20 nanofibers in fiber direction performed with a maximum Hencky strain $\varepsilon_H^{\text{max}} = 0.4$ for 9 cycles. For better visualization, the color of the symbols is changed from red to blue in a rainbow fashion as the experiment proceeds.
**Fig.S14. Mechanical properties of regenerated urethras.** (A) The stress-strain curve of the regenerated urethra film in each group. (B) The average modulus of the regenerated urethra in each group. $p<0.01$. (C) Elongational test of one piece of a regenerated urethra film at $\varepsilon_H=0.4$ on the elongational rheometer inset (the samples outside the clamps – not in front of the drums - are not deformed and do not contribute to the measurement).
Fig. S15. GPC diagrams of PU-alt block copolymers. E4-alt-C20 ($M_w=8.4 \times 10^4$, PDI=1.56). E10-alt-C20 ($M_w=14 \times 10^4$, PDI=1.35), and E20-alt-C20 ($M_w=9.5 \times 10^4$, PDI=1.61).
**Fig. S16.** Histological images of cross-sections of the rabbit represented hollow organ. Sirius red staining of (A) rabbit aorta, (B) rabbit jugular vein, (C) adult healthy rabbit urethra. Red (smooth muscle tissue), Yellow (Epithelial tissue), Purple black (elastin). Scale bar, 200 μm.

**Table. S1** Mechanical properties of PCL and PU-\(alt\) copolymers in wet state.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(R^a)</th>
<th>(E) (GPa)(^b)</th>
<th>(\delta) (MPa)(^c)</th>
<th>(\varepsilon) (%)(^d)</th>
</tr>
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<tbody>
<tr>
<td>PCL</td>
<td>-</td>
<td>0.32 ± 0.1</td>
<td>12.1 ± 1.3</td>
<td>120-700</td>
</tr>
<tr>
<td>E4-(alt)-C20</td>
<td>1:2:1</td>
<td>1.39 ± 0.02</td>
<td>12.6 ± 0.7</td>
<td>190-920</td>
</tr>
<tr>
<td>E10-(alt)-C20</td>
<td>1:2:1</td>
<td>1.19 ± 0.03</td>
<td>14.9 ± 0.4</td>
<td>200-1300</td>
</tr>
<tr>
<td>E20-(alt)-C20</td>
<td>1:2:1</td>
<td>0.21 ± 0.07</td>
<td>9.3 ± 0.2</td>
<td>130-1050</td>
</tr>
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</table>

\(a\): PCL-diol/HMDI/PEG molar ratio in feed.

\(b\): Young’s modulus.

\(c\): Stress at yield.

\(d\): Strain at break.
Sample abbreviation E4-\textit{alt}-C20 means that the feeding PEG segment $M_n = 0.4$ kDa; PCL-diol segment $M_n = 2.8$ kDa, and so on.

References
