Supporting information

Supplemental Figures



Fig. S1 Representative gross (A) and optical (B) images of aligned microfibers, nanofibers, and micro-nanofibers.



Fig. S2 (A) FFT output images of micro-, nano-, and micro-nanofibers. (B) Histograms representing angular distributions of aligned micro-, nano-, and micro-nanofibers.



Fig. S3 (A)Water contact angle photographs of the aligned micro-, nano-, and micronano porous PLLA fiber membranes taken in the directions parallel and perpendicular to the fiber axis with a video microscope. (B) Histogram shows the water contact angle data measured with the different fiber samples, n = 6. Data shown from representative sample and presented as mean \pm SD, **p < 0.01. One-way analysis of variance (ANOVA).



Fig. S4 F-actin staining images showed TSPCs adhered to the fibrous scaffolds and showed aligned morphology in each layer within aligned fibrous scaffolds. Scale bars, $100 \mu m$.



Fig. S5 Quantification of TGF β 1, TGF β 2, and pSMAD2/3 protein expression in the TSPCs cultured on flat surface or aligned fibrous scaffolds with or without RepSox treatment.



Fig. S6 (A) A gap wound was created and the Achilles tendon was removed to create a defect of 6 mm in length. Scaffolds were placed within the tendon defect and sutured to the tendon. (B) Gross view of neotendon at 2- and 4-weeks post-surgery.



Fig. S7 H&E staining showed the interaction between scaffold and regenerated tissue. Arrows indicated the remaining fibrous scaffolds. Scale bars, 200 µm.



Fig. S8 Repaired rat Achilles tendon at 2 weeks post-surgery. (A) Histological results of repaired rat Achilles tendon in section of Con, Micro, Nano, and Micro-nano groups at 2 weeks post-surgery. H&E staining and polarized light microscopy showing the

collagen fibrils at 2 weeks after implantation. Scale bars, 50 μ m (H&E) and 100 μ m (polarized light). Masson's trichrome staining showing the deposited collagen at the repaired tissue site. Scale bars, 50 μ m. (B) The maturation of repaired tendon was assessed by histology scoring, n = 4. (C) Quantitative analyses of the collagen content, n = 4. (D) Gene expression levels of tendon-related genes were assessed by real-time PCR. Data is represented as fold change relative to the Con group, set at 1, n = 4. Data shown from representative sample and presented as mean ± SD, **p* < 0.05, ***p* < 0.01. One-way analysis of variance (ANOVA).

Supplemental Table

Table S1. The electrospinning parameters used for the production of aligned microand nanofibers.

Scaffold	Applied voltage (kV)	Solution feeding rate (mL/h)	Collecting distance (cm)	Drum rotating speed (rpm)	Ambient temperature (°C)	Ambient humidity (%)
Microfibers	7	1	10	1500	20-25	40-60
Nanofibers	7	0.3	31	2000	20-25	40-60

Genes	5'-3'	Sequences	Production
			size (bp)
Gapdh	Forward	GCAAGTTCAACGGCACAG	141
	Reverse	CGCCAGTAGACTCCACGAC	
Scx	Forward	GCGAGAACACCCAGCCCAAAC	288
	Reverse	AAGCCATCACCCGCCTGTCCAT	
Mkx	Forward	CCCCGGACATCGGATCTACTA	300
	Reverse	CTCTTAGGATGAGGATTTAGGTA	
Egr1	Forward	CAGCGCCTTCAATCCTCAAG	78
	Reverse	GCGATGTCAGAAAAGGACTCTGT	
Collal	Forward	TGGATGGCTGCACGAGT	177
	Reverse	TTGGGATGGAGGGAGTTTA	
Tgfb1	Forward	GTGGCTTCTAGTGC	133

Table S2. Primer sequences of mouse specific genes used for QPCR.

Tgfb2ForwardTCGACATGGATCAGTTTATGCG147ReverseCCCTGGTACTGTTGTAGATGGA76DcnForwardAGACTCACAGCCGAGTAGGA376ReverseACATTCGCATCTCAGACACC152		Reverse	GCCTTAGTTTGGACAGGATCTG	
ReverseCCCTGGTACTGTTGTAGATGGADenForwardAGACTCACAGCCGAGTAGGA376ReverseACATTCGCATCTCAGACACC152	Tgfb2	Forward	TCGACATGGATCAGTTTATGCG	147
DenForwardAGACTCACAGCCGAGTAGGA376ReverseACATTCGCATCTCAGACACC700TnmdForwardGGGTGGTCCCGCAAGTGAAGGTG152		Reverse	CCCTGGTACTGTTGTAGATGGA	
ReverseACATTCGCATCTCAGACACCTnmdForwardGGGTGGTCCCGCAAGTGAAGGTG152	Dcn	Forward	AGACTCACAGCCGAGTAGGA	376
Tnmd Forward GGGTGGTCCCGCAAGTGAAGGTG 152		Reverse	ACATTCGCATCTCAGACACC	
	Tnmd	Forward	GGGTGGTCCCGCAAGTGAAGGTG	152
Reverse GCCTCGACGACAGTAAATACAACAGT		Reverse	GCCTCGACGACAGTAAATACAACAGT	
Hoxall Forward GTCTTCCGGCCACACTGAGG 160	Hoxa11	Forward	GTCTTCCGGCCACACTGAGG	160
Reverse CATGCGGGACAGTTGCAGACG		Reverse	CATGCGGGACAGTTGCAGACG	

 Table S3. Primer sequences of rat specific genes used for QPCR.

Genes	5'-3'	Sequences	Production
			size (bp)
Gapdh	Forward	GCAAGTTCAACGGCACAG	141
	Reverse	CGCCAGTAGACTCCACGAC	
Scx	Forward	GCGAGAACACCCAGCCCAAAC	288
	Reverse	AAGCCATCACCCGCCTGTCCAT	
Mkx	Forward	CCCCGGACATCGGATCTACTA	300
	Reverse	CTCTTAGGATGAGGATTTAGGTA	
Egr1	Forward	CAGCGCCTTCAATCCTCAAG	78
	Reverse	GCGATGTCAGAAAAGGACTCTGT	
Col1a1	Forward	TGGATGGCTGCACGAGT	177
	Reverse	TTGGGATGGAGGGAGTTTA	
Tgfb2	Forward	TCGACATGGATCAGTTTATGCG	147
	Reverse	CCCTGGTACTGTTGTAGATGGA	
Dcn	Forward	AGACTCACAGCCGAGTAGGA	376
	Reverse	ACATTCGCATCTCAGACACC	
Tnmd	Forward	GGGTGGTCCCGCAAGTGAAGGTG	152
	Reverse	GCCTCGACGACAGTAAATACAACAGT	

Runx2ForwardCCAACTTCCTGTGCTCCGTG143ReverseTAAGTAAAGGTGGCTGGATAGT