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Supplementary Materials for

Functional regeneration of tendons using scaffolds with physical anisotropy engineered via microarchitectural manipulation

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/4/10/eaat4537/DC1)

SUPPLEMENTARY MATERIALS

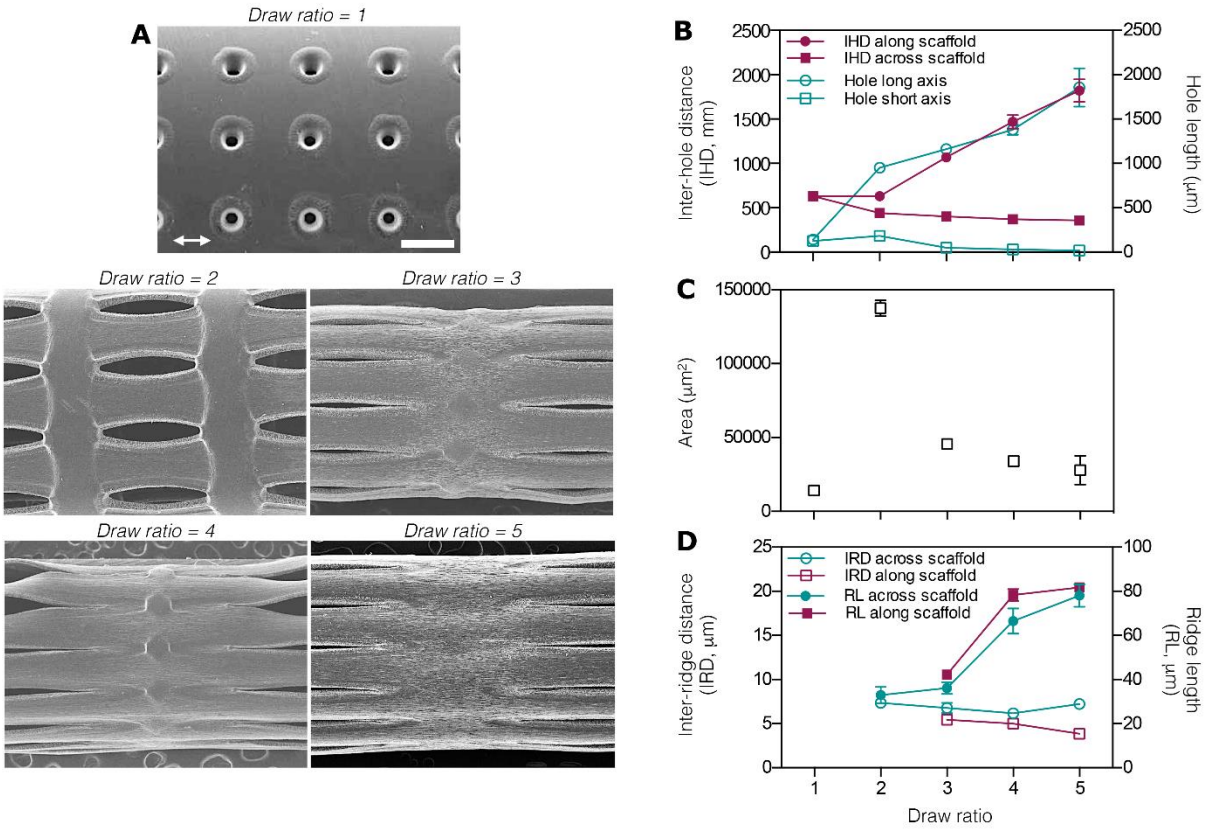

Fig. S1. Polymer axial drawing for through-hole expansion and microridge/groove propagation. (**A**) Representative SEM images of PCL tubes showing a dynamical structure formation during polymer deformation. Scale bar, 500 μm. (**B**) Inter-hole distance (IHD, N = 20) and hole size (N = 8~20) showing PCL tube elongation as a result of the increase in IHD and hole length along stretching direction, and PCL tube necking associated with the reduction in IHD and hole width across stretching direction. (**C**) Quantitative measurement of through-hole area as a function of draw ratio (N = 8~20). (**D**) Inter-ridge distance (IRD, N = 60) and ridge length (RL, N = 40) coupling with shoulder propagation during polymer axial-drawing. Error bars represent s.d..

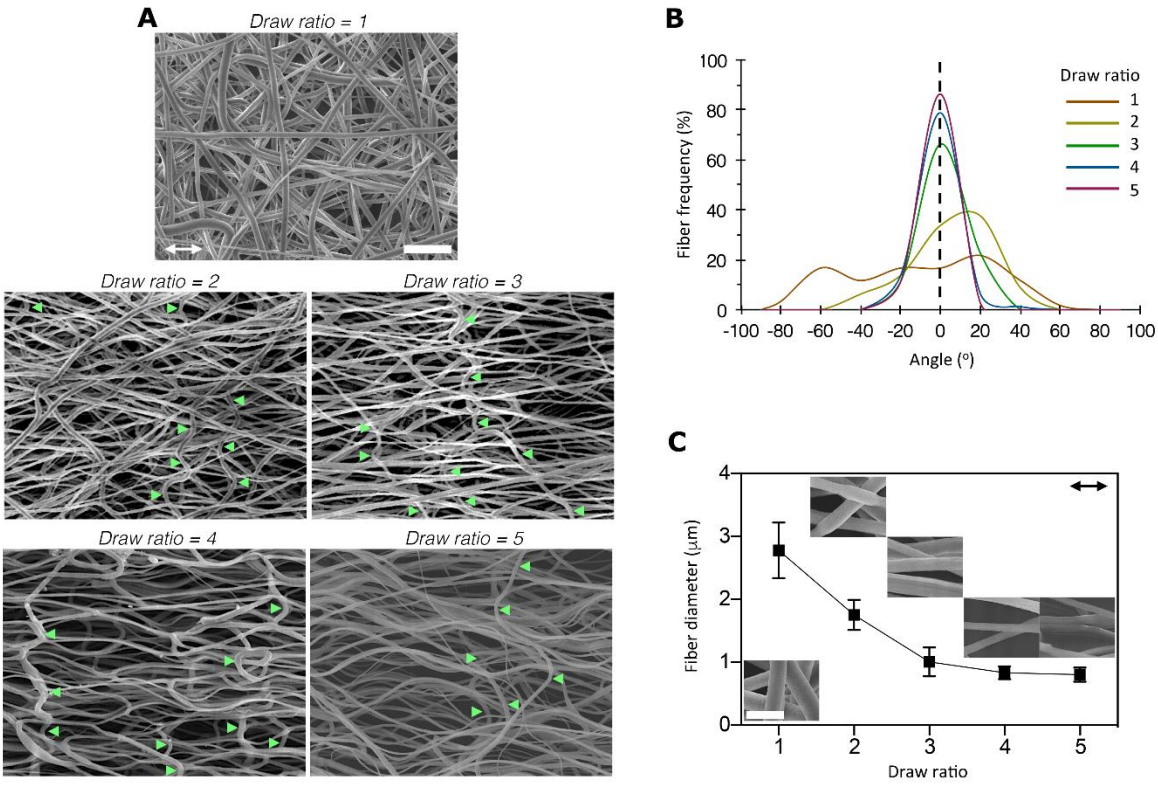

Fig. S2. Polymer axial drawing for fiber reorientation and deformation. (**A**) Representative SEM images of PCL fibre mesh showing a fibre morphological evolution during polymer drawing. Green arrow, loose fibres; and double-headed arrow, stretching direction. Scale bar, 200 μm. (**B**) Representative fibre angular frequency curves showing progressive reorientation of fibres towards stretching direction (dashed line). (**C**) Quantitative measurement of fibre diameter as a function of draw ratio (N = 80). Inset, representative SEM images of fibres at draw ratios from 1 to 5; and double-headed arrow, stretching direction. Scale bar, 5 μm. Error bars represent s.d..

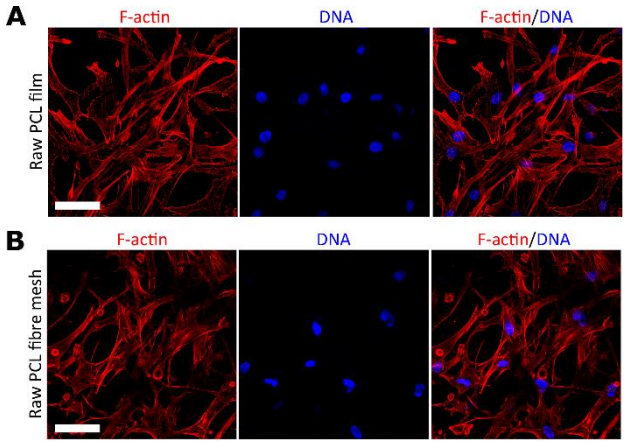

Fig. S3. Cytoskeletal organization and nucleus morphology of human tenocytes. Representative confocal fluorescence images of human tenocytes after 14 days of culturing with raw PCL film (**A**, control of shell portion) and fibre mesh (**B**, control of core portion). Red color, F-actin; and blue color, DNA. Scale bar, 50 μm.

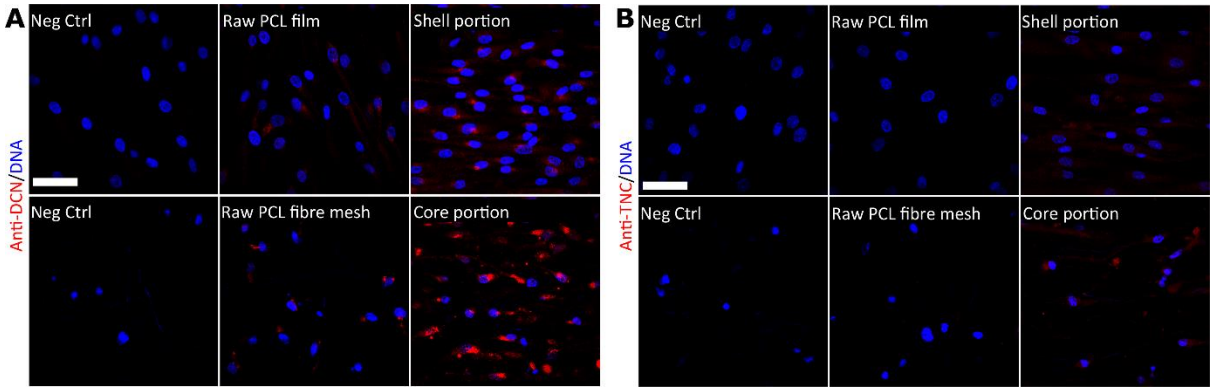

Fig. S4. Human tenocytes to express minor tendon matrix proteins. Representative confocal fluorescence images showing elevated expression of DCN (**A**) and TNC (**B**) for cells after 7 days of culturing with shell (vs. control of raw PCL film) and core (vs. control of raw PCL fibre mesh) portions. DCN, decorin; TNC, tenascin-c; and Neg Ctrl, negative control. Red color, tenogenic marker; and blue color, DNA. Scale bar, 50 μm.

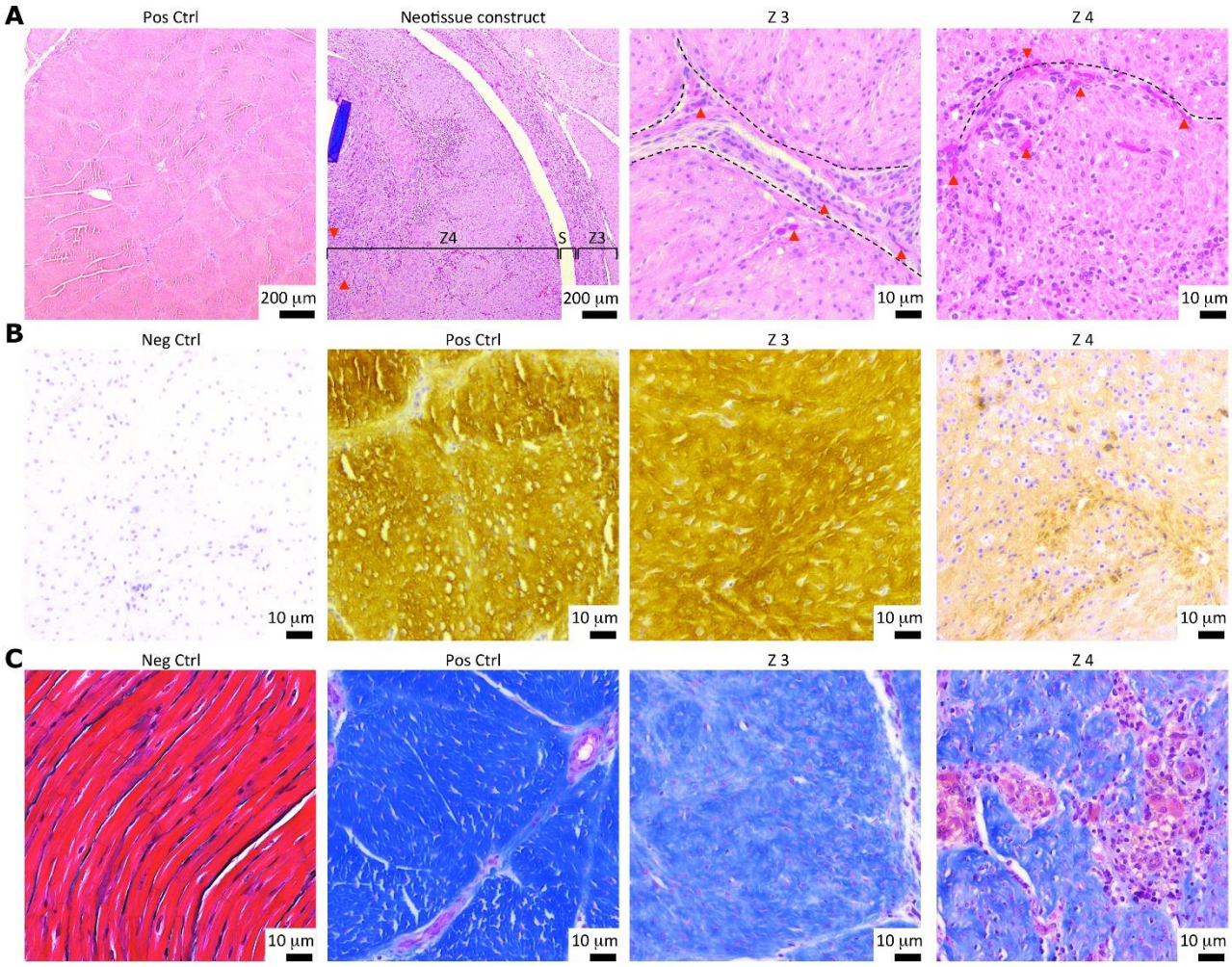

Fig. S5. Cross section of tendon neotissue construct. (**A**) Haemotoxylin and eosin (H&E) images showing full neotissue formation of the scaffold 3 months post operation. s, scaffold edge; z3 and z4, zone of shell and core portions of implanted scaffold; and Pos Ctrl, positive control of native tendon tissue. Red arrow, blood cells and vessels; and dashed line, compartmentalization. (**B**) Immunohistological images showing positive deposition of COL-I matrix in the scaffold. COL-I, collagen type I; Neg Ctrl, negative control of isotype IgG1; and Pos Ctrl, positive control of native tendon tissue. Yellow colour, COL-I; and blue colour, cell nuclei. (**C**) Masson's trichrome images showing fibrous matrix formation in the scaffold, with compartments of hypo- and hyper-cellular regions. Neg Ctrl, negative control of heart tissue; and Pos Ctrl, positive control of native tendon tissue. Blue colour, matrix fibres; red/pink colour, cytoplasm; and dark/purple colour, nuclei.

Table S1. Mechanical properties of thermally stretched PCL film tube. (n=4)

Samples	Young's modulus (MPa)	Yield stress (MPa)	Yield strain (%)	Ultimate stress (MPa)	Break strain (%)
PCL film tube	438.9 ± 37.9	46.4 ± 4.4	11.8 ± 2.0	114.8 ± 8.6	182.7 ± 9.0

*: Fabricated from raw film tube using polymer axial-drawing at a draw ratio of 5 and temperature of 54 ^{o}C.

Table S2. Compiled list of monoclonal antibodies targeted for human tendon matrix markers.

Primary monoclonal antibodies (Mouse anti-human)	IgG isotypes	Dilution in 1 wt.% BSA solution (v/v)
COL-I (COL1A1)	IgG 1	1:100
TNC	IgG 1	1:100
DCN	IgG 1	1:100
Second antibody (Goat anti-mouse IgG (H+L)-Alexa Fluor® 594)	/	1:200