

Supplementary Information

Title: Application of a bilayer tubular scaffold based on electrospun poly(l-lactide-co-caprolactone)/collagen fibers and yarns for tracheal tissue engineering

Authors: *Tong Wu, Hui Zheng, Jianfeng Chen, Yuanfei Wang, Binbin Sun, Yosry Morsi, Hany EI-Hamshary, Salem S. Al-Deyab, Chang Chen, Xiumei Mo*

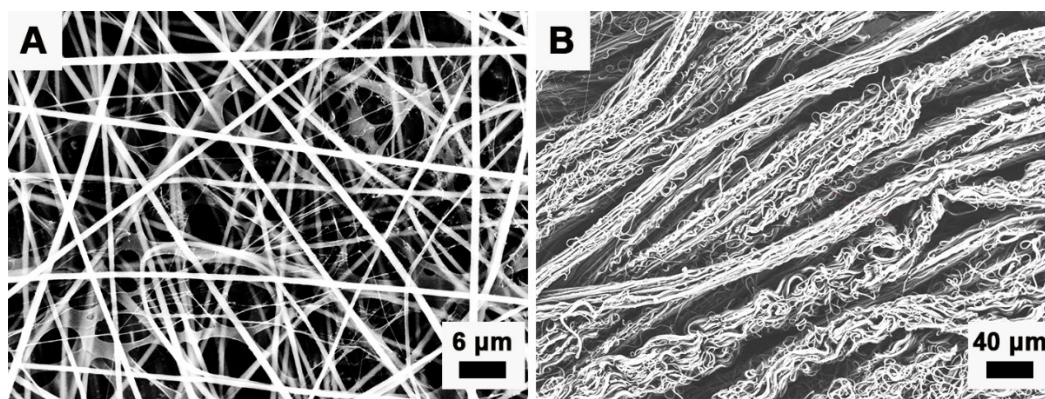


Figure S1. SEM images of (A) collagen/P(LLA-CL) fibers (FS-CP) and (B) collagen/P(LLA-CL) yarns (YS-CP) after crosslinking.

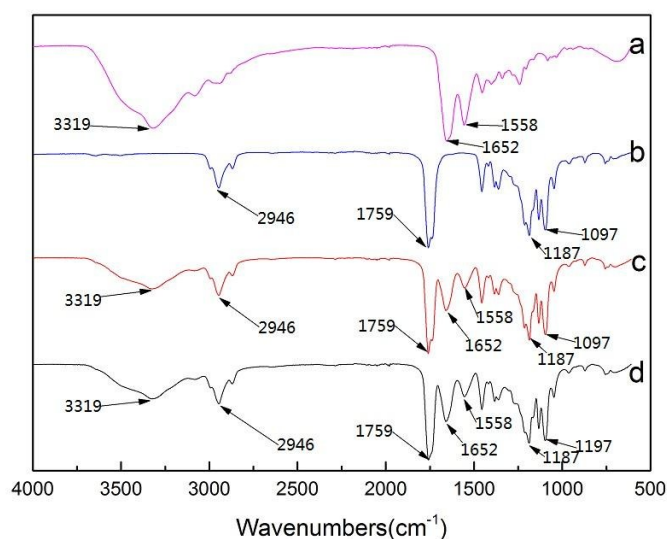


Figure S2. Fourier transform infrared spectroscopy (FTIR) of (a) collagen, (b) P(LLA-CL), (c) collagen/P(LLA-CL) fibers (inner layer) and (d) collagen/P(LLA-CL) yarns (outer layer). (Ref: J. Chen, W. Liu, T. Wu, D. Li, J. Zhang, N. WANG and X. Mo, *Journal of Donghua University (Eng. Ed.)*, 2014, **31**, 718-722.)

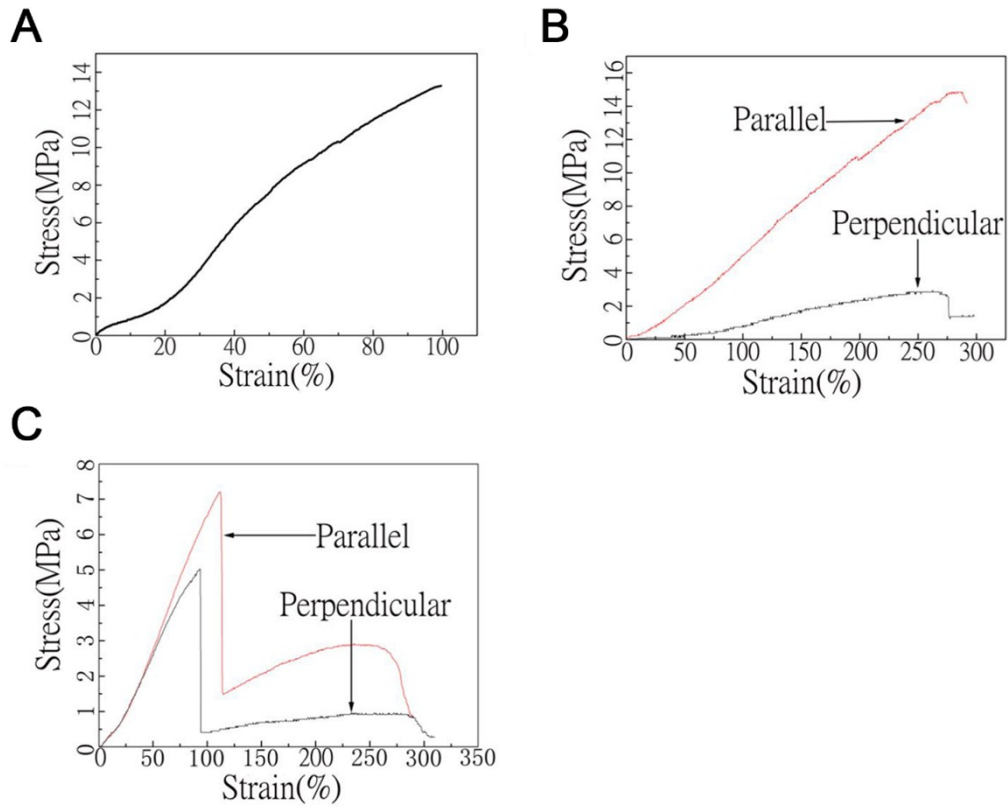


Figure S3. Typical stress-strain curves of (A) fibers inner layer, (B) yarns outer layer and (C) the bilayer scaffold. (Ref: J. Chen, W. Liu, T. Wu, D. Li, J. Zhang, N. WANG and X. Mo, *Journal of Donghua University (Eng. Ed.)*, 2014, **31**, 718-722.)

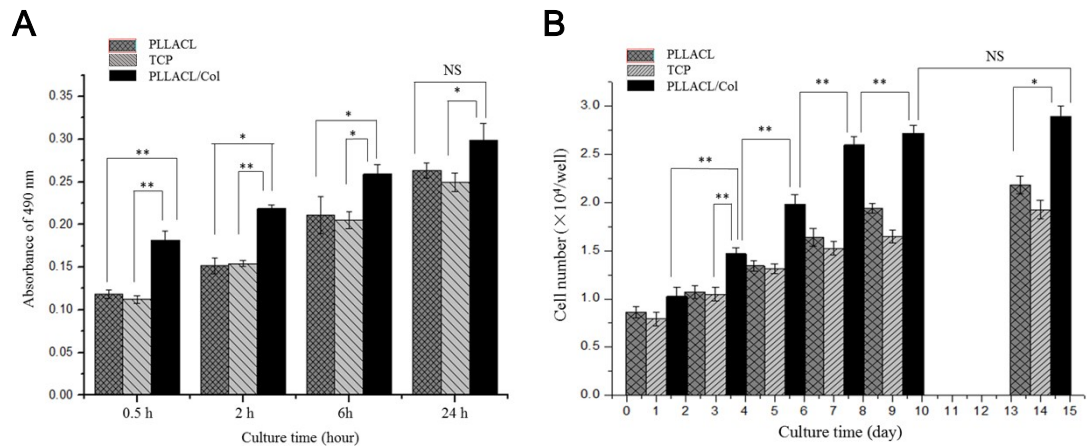


Figure S4. Tracheal epithelial cells (RTECs) adhesion (A) and proliferation (B) on P(LLA-CL) fibers (FS-P) and collagen/P(LLA-CL) fibers (FS-CP) by MTT methods (* indicated significant difference at $P < 0.05$, ** indicated highly significant difference at $P < 0.001$ and NS indicated no significant difference).

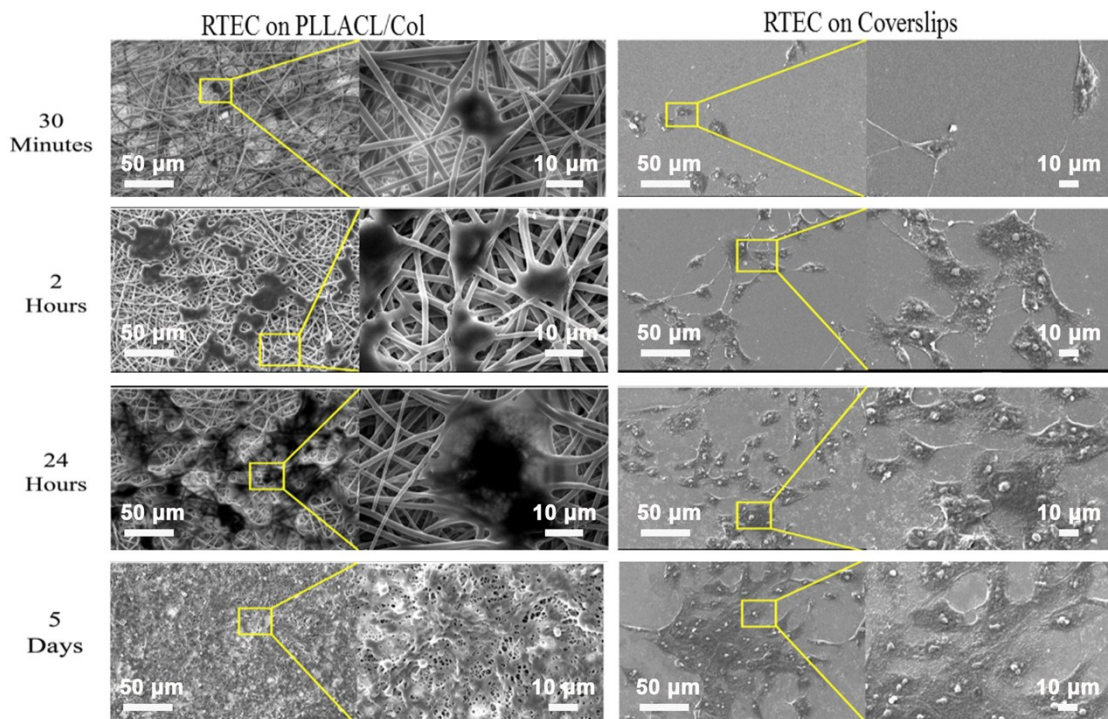


Figure S5. SEM images of RTECs growth on collagen/P(LLA-CL) fibers after culturing for 30 minutes, 2 hours, 24hours, and 5 days with coverslips as comparison.

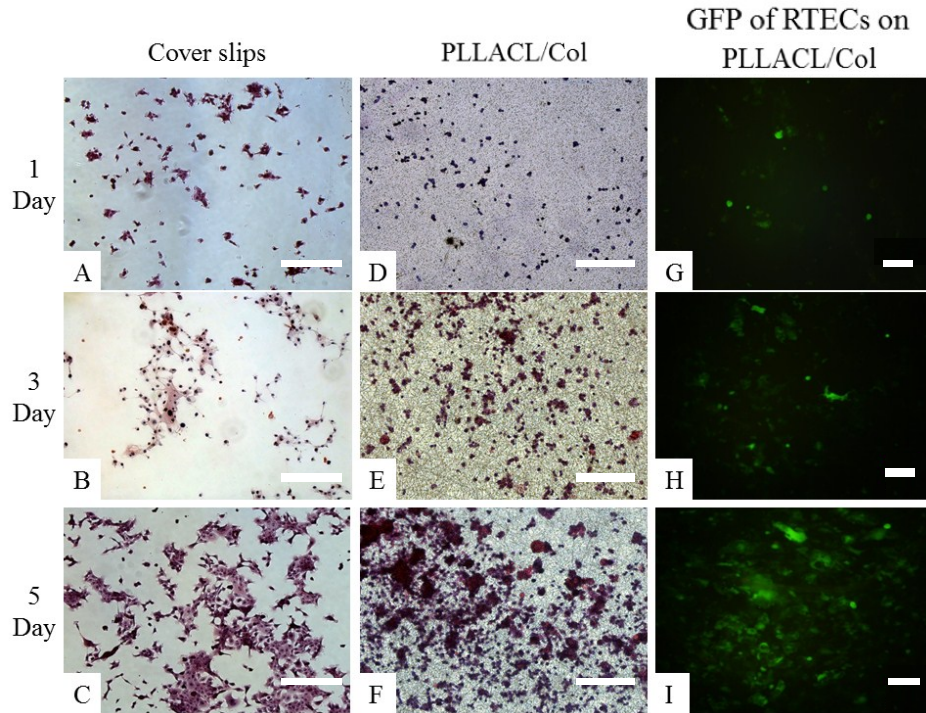


Figure S6. (A-F) H&E images of RTECs morphology and density on collagen/P(LLA-CL) fibers after culturing for 1, 3, and 5 days with coverslips as comparison; (G-I) Fluorescent images of green fluorescent protein (GFP) expression of RTECs on collagen/P(LLA-CL) fibers after gene transfection, bar = 100 μ m.

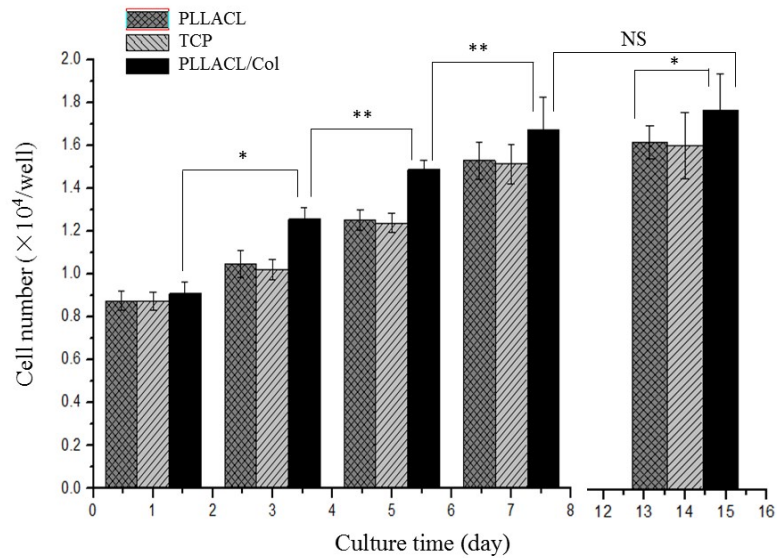


Figure S7. Tracheal chondrocytes proliferation on P(LLA-CL) yarns (YS-P) and collagen/P(LLA-CL) yarns (YS-CP) by MTT methods (* indicated significant difference at $P < 0.05$, ** indicated highly significant difference at $P < 0.001$ and NS indicated no significant difference).

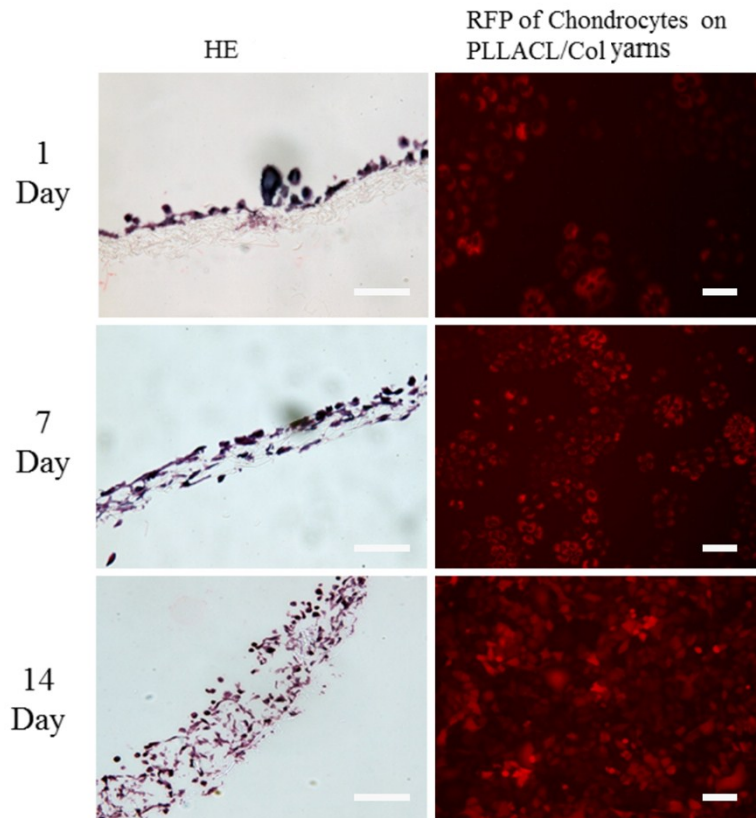


Figure S8. H&E images of tracheal chondrocytes penetration on collagen/P(LLA-CL) yarns after culturing for 1, 7 and 14 days; (G-I) Fluorescent images of red fluorescent protein (RFP) expression of tracheal chondrocytes on collagen/P(LLA-CL) yarns after gene transfection, bar = 100 μm .

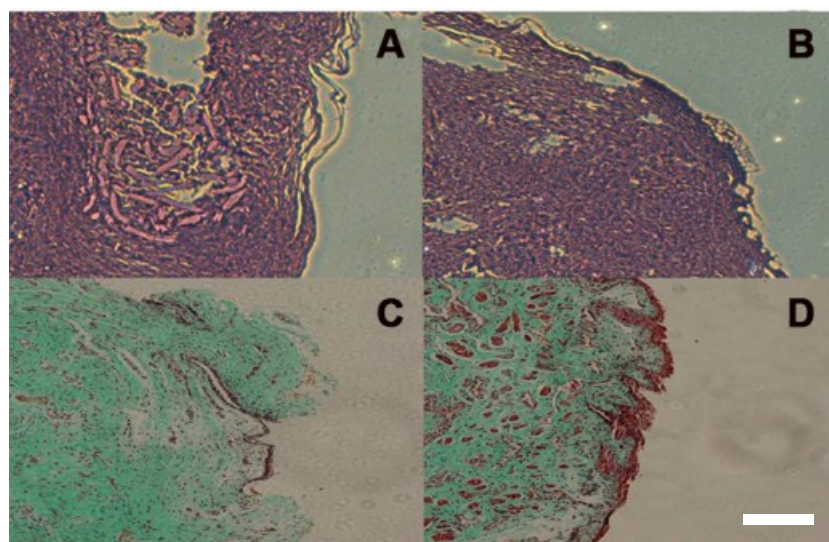


Figure S9. H&E staining (A, B) and Masson staining (C, D) images of cells penetration into Collagen/P(LLA-CL) yarns after subcutaneous embedding for 2 weeks (A, C) and 4 weeks (B, D), bar = 100 μm .



Figure S10. Digital photos of BTLS after cells-seeding, bar = 1 cm.

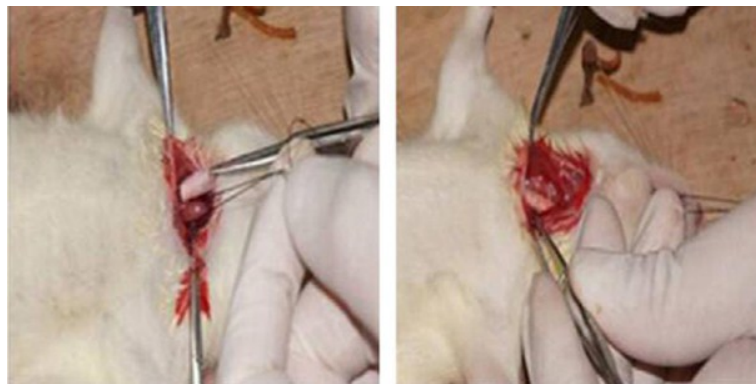


Figure S11. Images of BTLS for pre-vascularization processing.



Figure S12. Surgical images of BLTS *in situ* implantation in a rat tracheal.

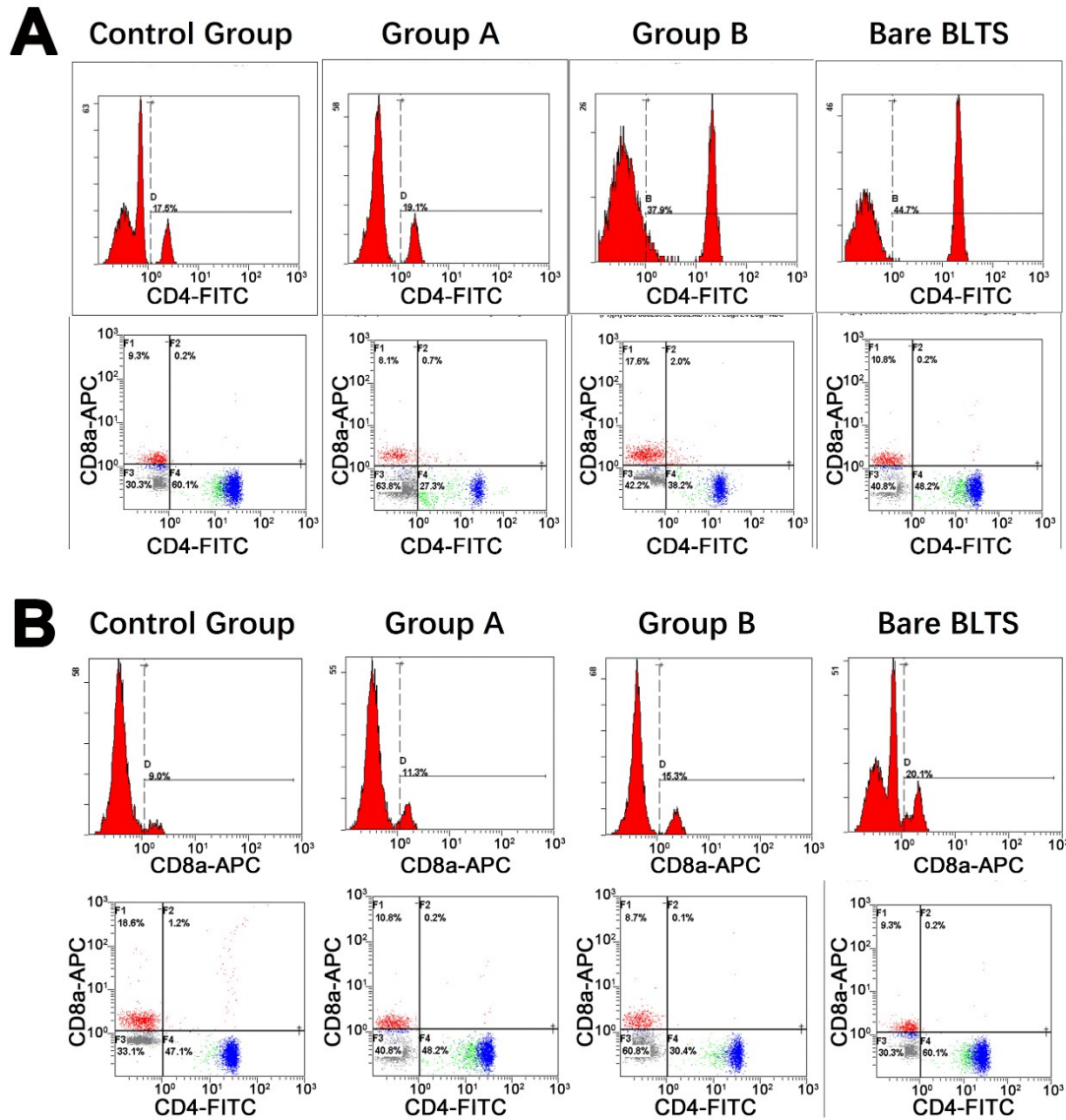


Figure S13. Flow cytometry analysis of (A) CD4T and (B) CD8T in rat peripheral blood after implantation for 2 weeks.

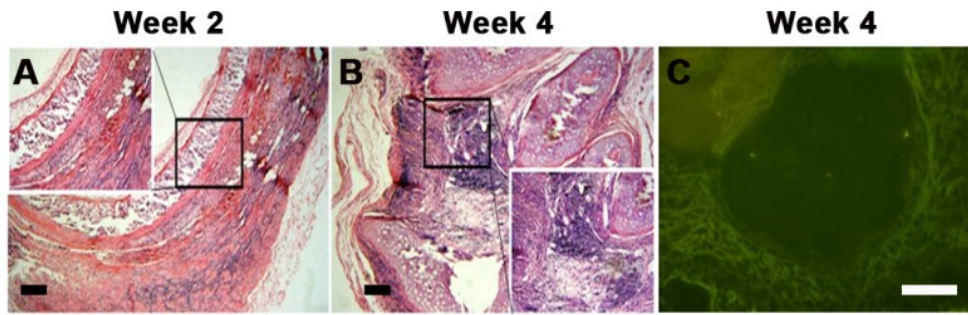


Figure S14. H&E staining images of cell growth and lumen morphology (A, B), and immunofluorescence images with anti-cytokeratin antibody (C) of bare BLTS after *in vivo* implantation, bar = 100 μm .