

RESEARCH ARTICLE

Primitive regenerated-tissue evolution *in situ* after implantation of a 3D-printed tracheal graft

Supplementary File

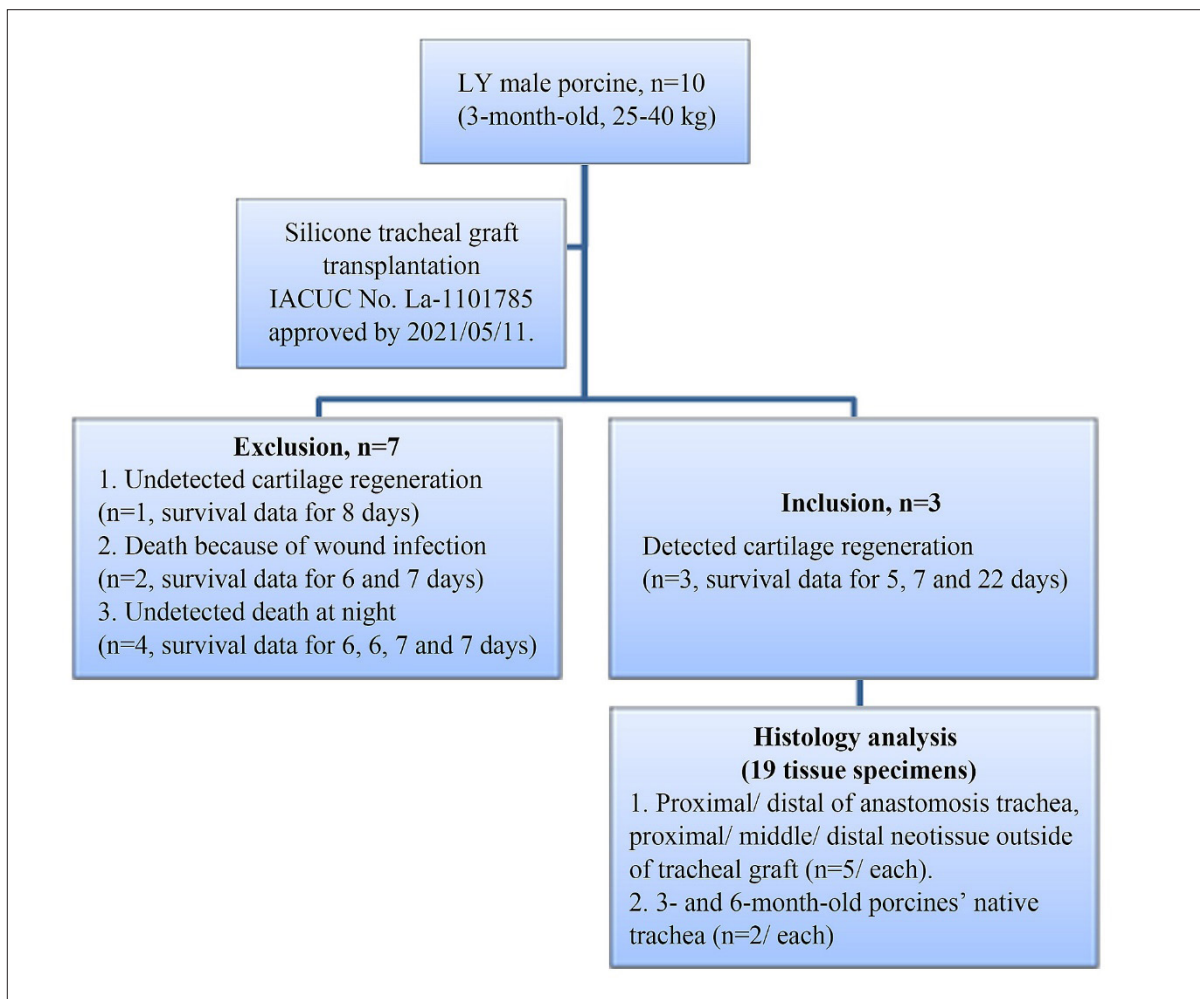


Figure S1. A schematic diagram illustrating the experimental design involving animal subjects.

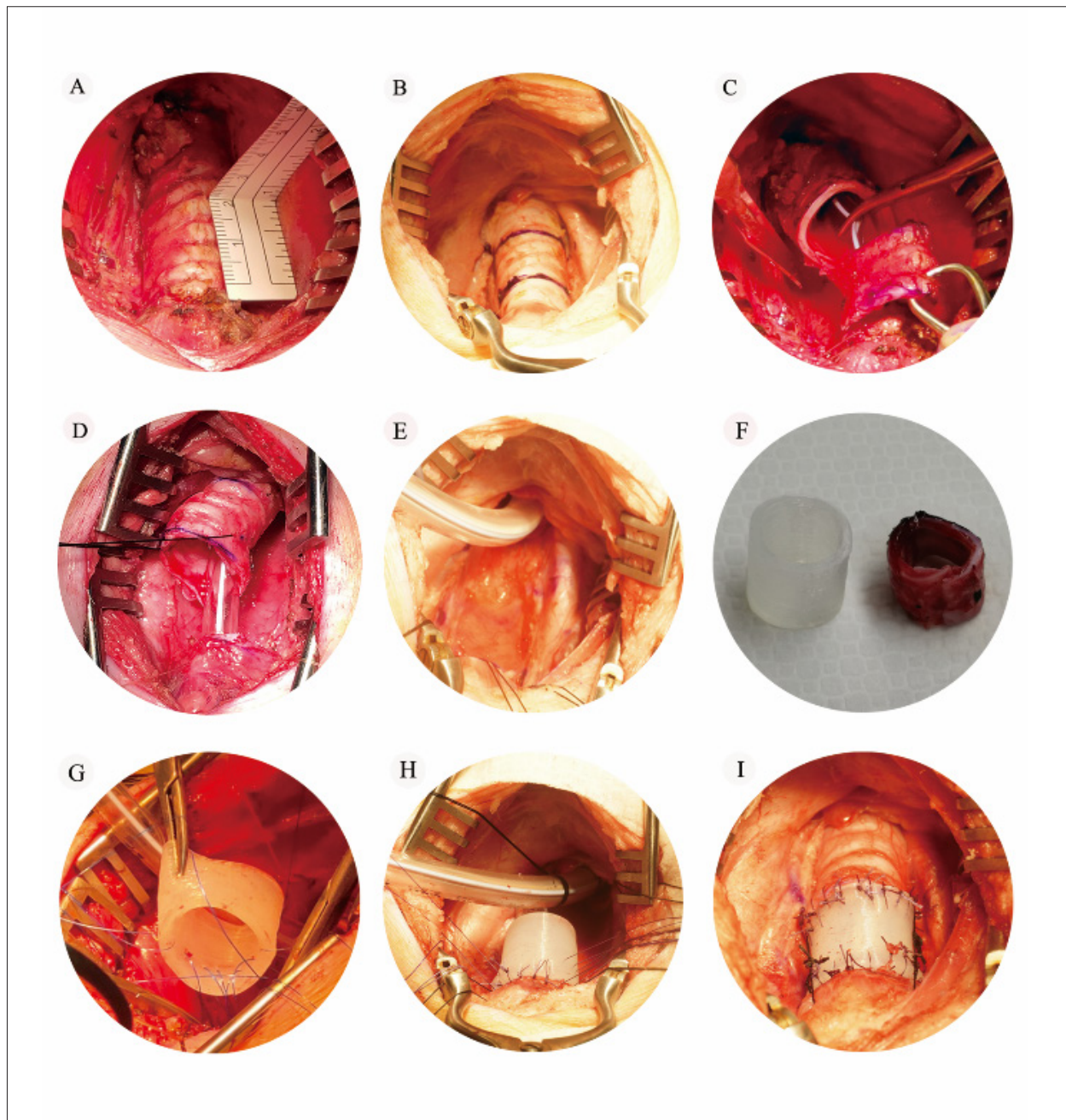


Figure S2. Procedures for surgical intervention for tracheal graft transplantation in a porcine model. (A, B) Making a 2-cm-long mark on the native trachea for resection. (C) Resected tracheal segment. (D, E) Traction sutures with proximal and distal tracheal. (F) Silicone graft compared with native trachea. (G, H) Anastomosis of the proximal end of the trachea with graft. (I) Completion of graft reconstruction.

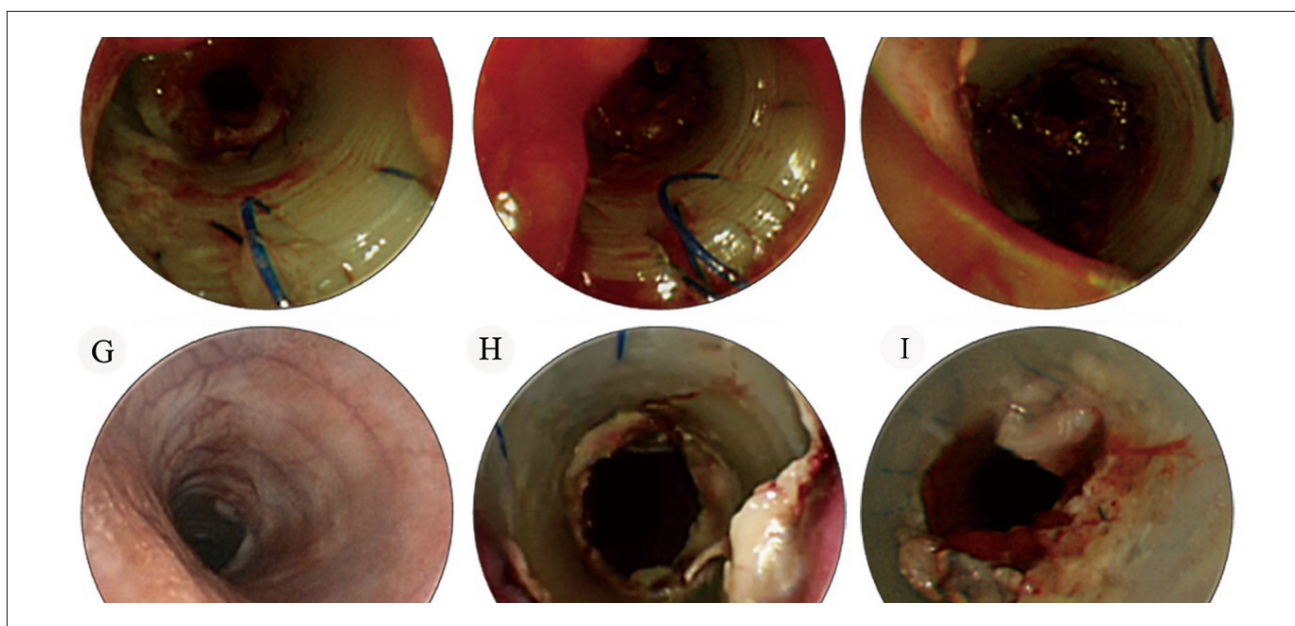


Figure S3. Bronchoscopy examination before and after tracheal graft transplantation. (A and G) Normal trachea of a 3-month-old porcine before transplantation. (B–F) Proximal tracheal anastomosis examination via bronchoscopy on days 5 (B), 8 (C), 13(D), 16 (E), and 19 (F) after transplantation. (H–L) Distal tracheal anastomosis examination via bronchoscopy on days 5 (H), 8 (I), 13 (J), 16 (K), and 19 (L) after transplantation.

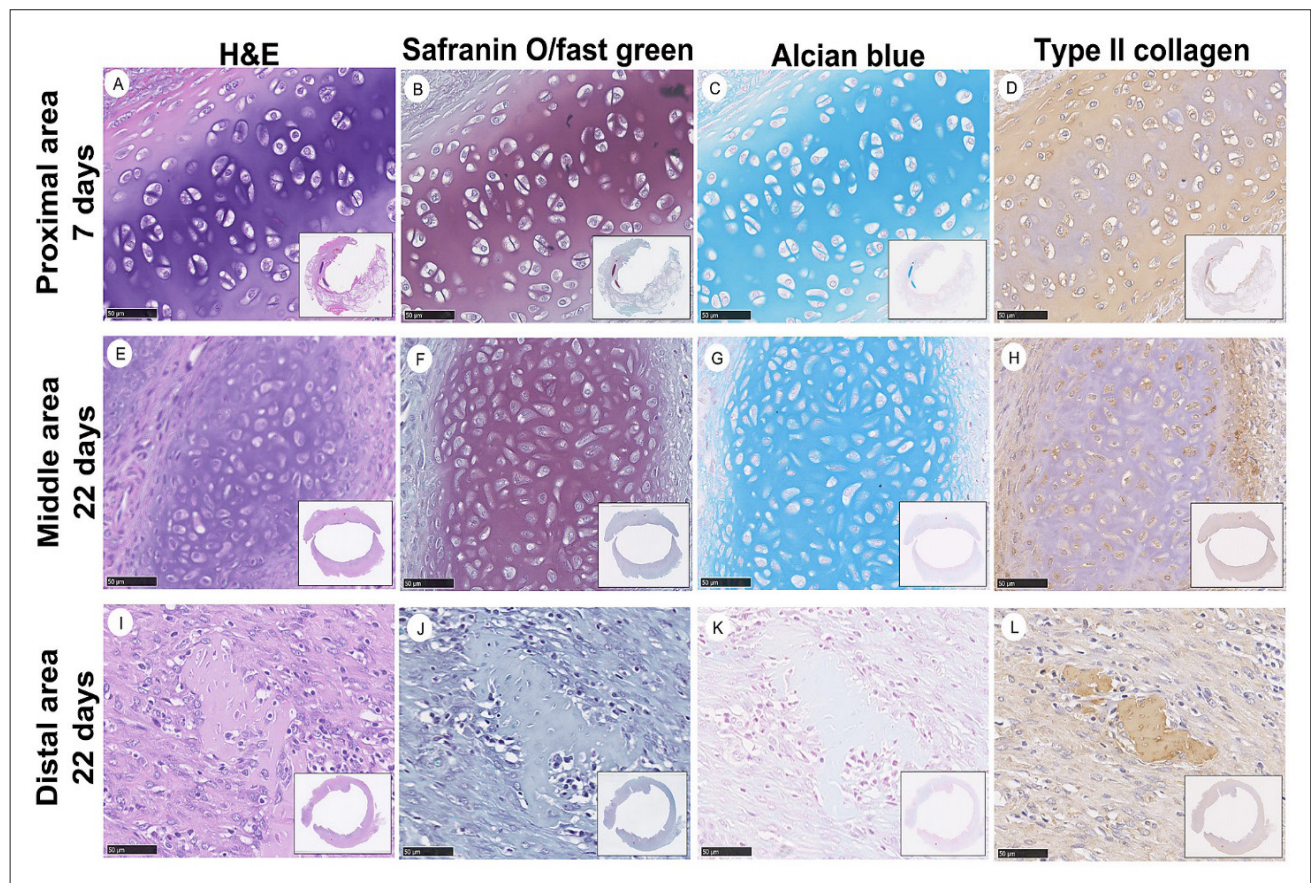


Figure S4. Evaluation of initial chondrogenesis in the proximal, middle, and distal areas outside the graft neotissue. (A–D) Chondrogenesis within the proximal area of tubular graft tissue at 7 days post-transplantation. (E–H) Chondrogenesis within the middle area of tubular graft tissue at 22 days post-transplantation. (I–L) Chondrogenesis within the distal area of tubular graft tissue at 22 days post-transplantation. Neocartilages were investigated by hematoxylin and eosin (H&E; A, E, and I), safranin O/fast green (B, F, and J), alcian blue (C, G, and K), and immunohistochemistry (IHC; type II collagen; D, H, and L) stains. Type II collagen protein expression is indicated in brown color; safranin O/fast green is indicated in red color; alcian blue is indicated in blue color. Magnification: 400 \times . Scale bars: 50 μ m.

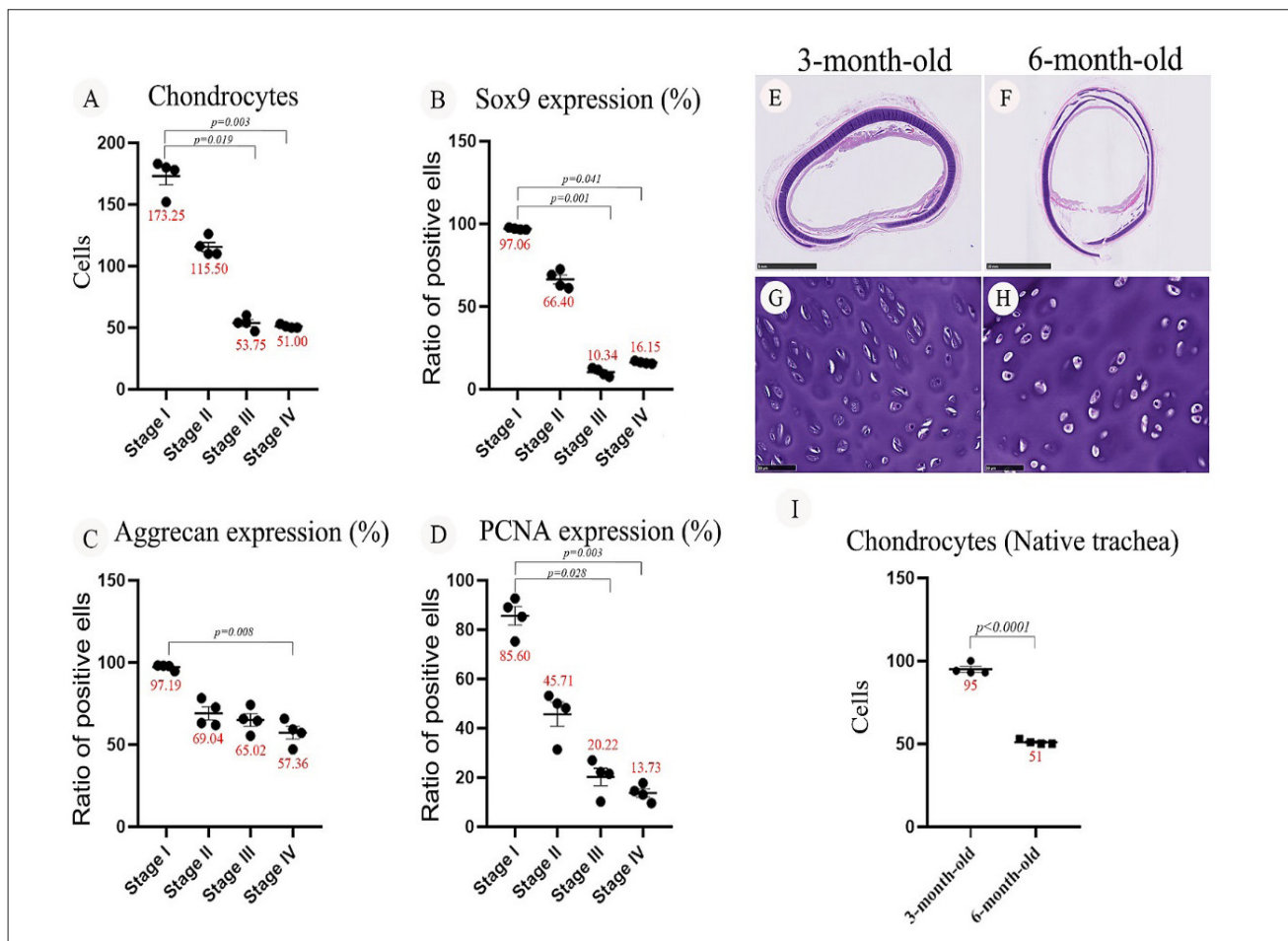


Figure S5. Statistical analyses of the proposed stages of chondrogenesis within the graft neotissue based on hematoxylin and eosin (H&E) and immunohistochemistry (IHC; Sox9, aggrecan, and PCNA antibodies) staining, as well as the shape of the tracheal lumen and chondrocyte morphology in 3- and 6-month-old native porcine trachea. (A) Chondrocyte number at different chondrogenesis stages of graft neotissue using H&E staining ($n = 4$; viewed at $400\times$ magnification); data are presented as the mean \pm standard error of the mean (SEM). Chondrocyte number in 6-month-old native trachea: 173.25 ± 7.16 (Stage I); 115.50 ± 3.78 (Stage 2); 53.75 ± 2.66 (Stage III), and 51.00 ± 0.71 (Stage IV). (B–D) The ratio of protein expression (Sox9 [B], aggrecan [C], and PCNA [D]) quantified at different chondrogenesis stages of neotissue outside the graft based on IHC staining ($n = 4$; viewed at $200\times$ magnification); data are presented as the mean \pm SEM; statistically significant differences across stages were evaluated at $p < 0.05$. (B) Sox9 expression: 97.06 ± 0.29 (Stage I); 66.40 ± 2.66 (Stage II); 10.34 ± 1.19 (Stage III); and 16.15 ± 0.41 (Stage IV). (C) Aggrecan expression: 97.19 ± 0.83 (Stage I); 69.04 ± 3.93 (Stage II); 65.02 ± 3.87 (Stage III); and 57.36 ± 3.86 (Stage IV). (D) PCNA expression: 85.60 ± 3.75 (Stage I); 45.71 ± 4.88 (Stage II); 20.22 ± 3.55 (Stage III); and 13.73 ± 1.68 (Stage IV). (E–H) Cartilage shape containing chondrocytes in 3- and 6-month-old native porcine trachea; magnification: $400\times$; scale bars: 5 mm in E–F and 50 μm in G–H. (E–I) the 3-month-old and 6-month-old native trachea were as a native control to compare with the image and data of neocartilage. (I) Chondrocyte number in 3- and 6-month-old native porcine trachea cartilage ($n = 4$, viewed at $400\times$ magnification); data are presented as the mean \pm SEM: 95.0 ± 1.68 (3-month-old) versus 51.0 ± 0.71 (6-month-old) ($p < 0.0001$). Abbreviation: PCNA, proliferating cell nuclear antigen.

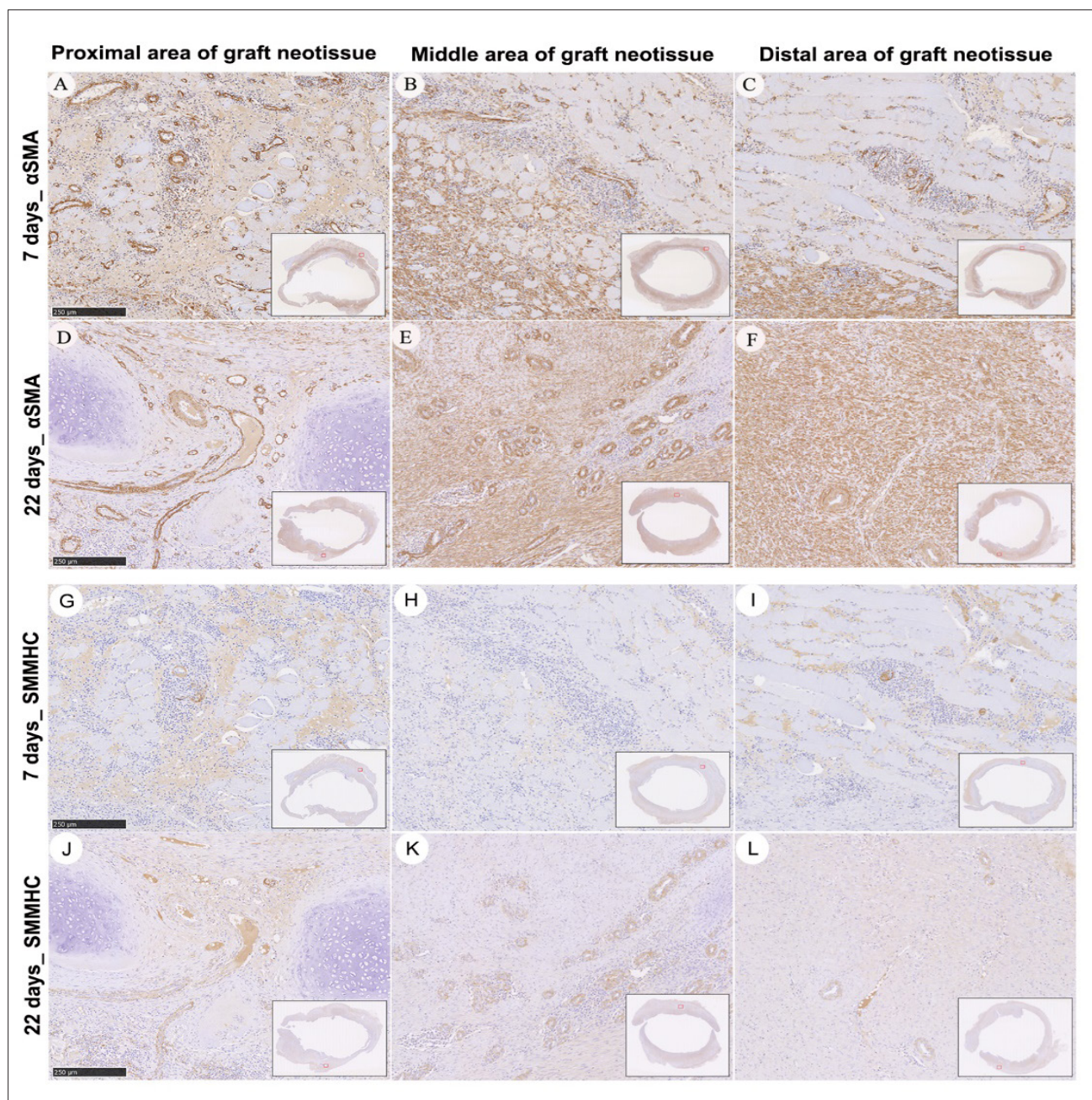


Figure S6. Protein expression (α SMA and SM-MHC) of neomuscle tissue in the proximal, middle, and distal areas outside the graft after tracheal graft implantation. (A–F) Early muscle cell marker (α SMA) displayed strong expression within the proximal, middle, and distal areas of graft tissue at 7 and 22 days post-transplantation. (G–L) Late muscle cell marker (SM-MHC) displayed a weak expression within the proximal, middle, and distal areas of graft neotissue at 7 and 22 days post-transplantation. The proteins were stained brown by immunohistochemistry (IHC) staining. Magnification: 100 \times . Scale bars: 250 μ m. Abbreviations: α SMA, alpha smooth muscle actin; SM-MHC, smooth muscle myosin heavy chain.

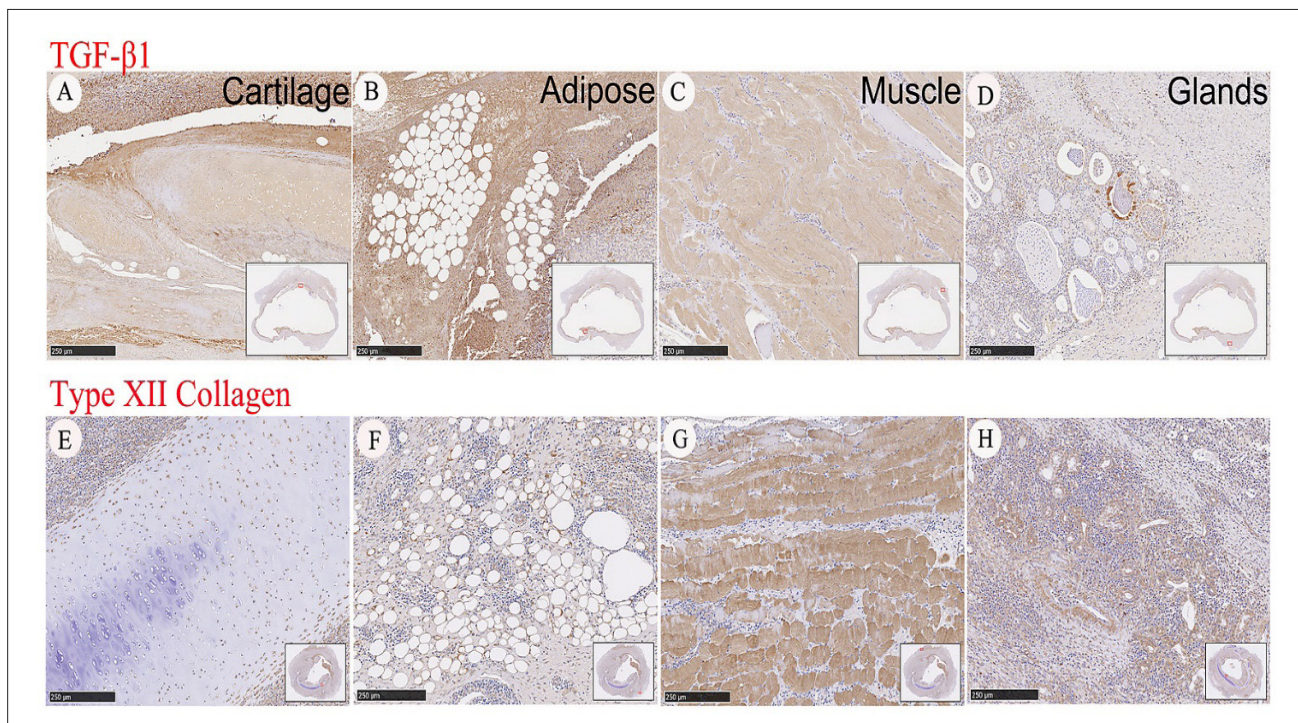


Figure S7. Detection of TGF- β 1 and type XII collagen in cartilage, adipose, muscle tissue, and glands at 7 days post-transplantation. (A–D) TGF- β 1 is expressed in neocartilage, neoadipose tissue, neomuscle, and neoglands. (E–H) Type XII collagen is expressed in neocartilage, neoadipose tissue, neomuscle, and neoglands. TGF- β 1 and type XII collagen protein expression are indicated by brown color. Magnification: 100 \times . Scale bars: 250 μ m. Abbreviation: TGF, transforming growth factor.

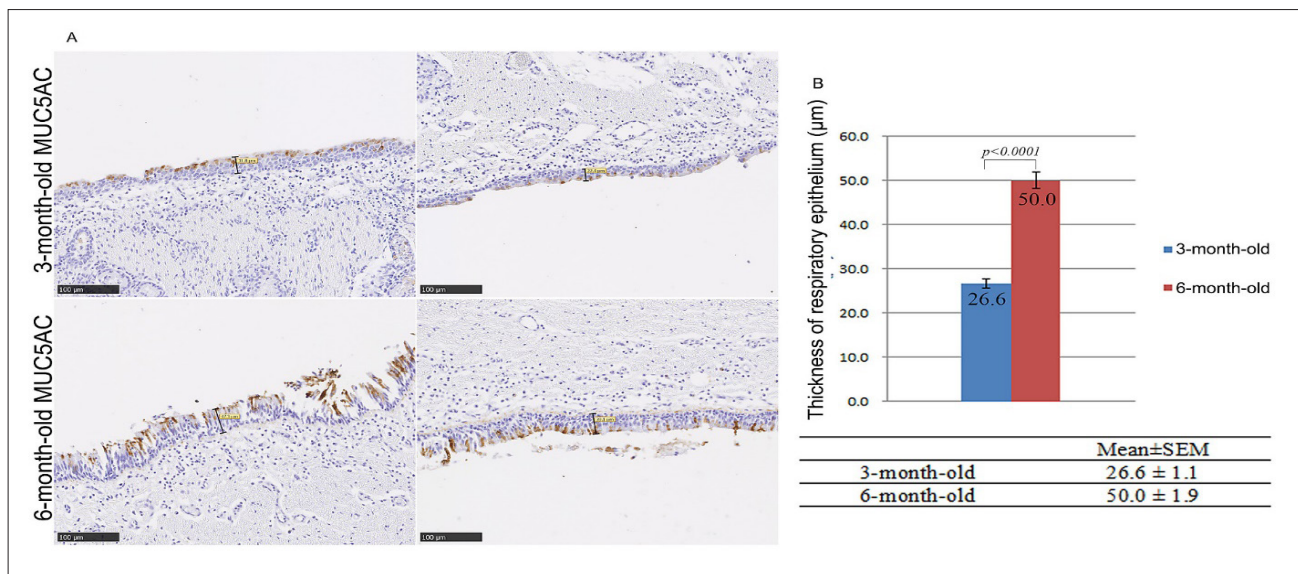


Figure S8. The thickness of native tracheal epithelium. (A) Comparison of the thickness of the respiratory epithelium and MUC5AC expression (brown) between the 3- and 6-month-old native trachea. (B) Thickness of the respiratory epithelium; data are presented as the mean \pm standard error of the mean (SEM): 26.6 \pm 1.1 (3-month-old) and 50.0 \pm 1.9 (6-month-old) ($p < 0.0001$). Magnification: 200 \times . Scale bars: 100 μ m.

Video

Video S1. Procedures for surgical intervention of silicone graft transplantation. (<https://drive.google.com/file/d/1nMJM3hqob2Ug1wbH8SE2h4oP2WO7tlvV/view?usp=sharing>).