Supplementary Information

Imaging-Guided Bioreactor for De-Epithelialization and Long-Term Cultivation of *Ex Vivo* Rat Trachea

Mohammad Mir, Jiawen Chen, Meghan R. Pinezich, John D. O'Neill, Sarah X. L. Huang, Gordana Vunjak-Novakovic, and Jinho Kim*

*Corresponding author
E-mail: jkim6@stevens.edu
Phone: 201-216-5518
Fax: 201-216-8196

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I. Supplementary Methods and Materials

1. Image processing of the mechanical vibration videos

High-frame rate imaging method was used to monitor the movement of the mechanical vibration stage. To process the videos, we used the methods described in our previous report[S1]. Briefly, videos recorded at 240 frame per second (fps) were stored in AVI file format which is a standard video file format compatible with ImageJ. To determine the displacement distance of the stage, a ruler was placed adjacent to the shaker as a reference object with known length. The video file was imported to ImageJ as a sequence of image frames using "AVI reader" plugin. The displacement distance of the sample stage in the image sequence was measured with respect to the reference ruler. In the meantime, to improve visibility of the stage movement, "enhance contrast" and "find edges" functions were used in ImageJ. To plot the displacement curves, a small region of the stage moving up and down was cropped and extracted from each image frame. The cropped images were then stitched horizontally with "grid/collection stitching" plugin as a single image file. The similar procedure was done for different regions of the stage to generate a continuous waveform that represent displacement of the sample stage over time.

II. Supplementary Figures

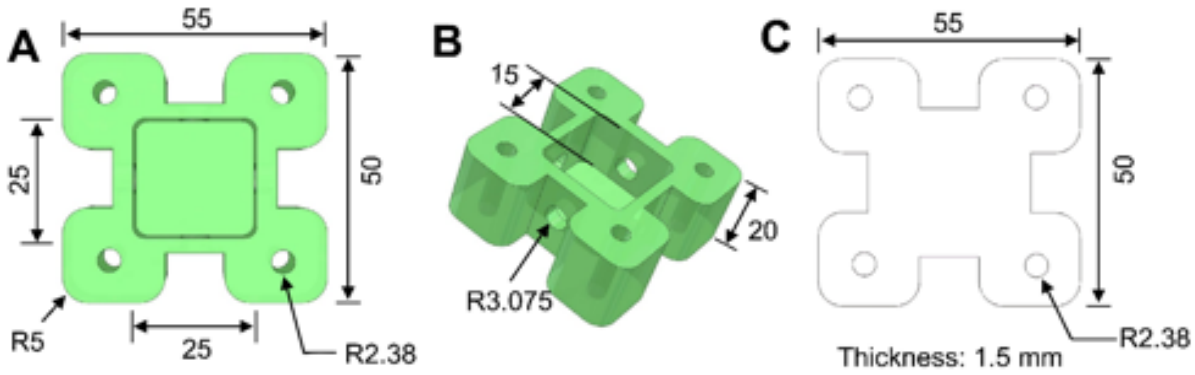


Fig. S1. (**A, B**) Dimensions of the bioreactor chamber. (**C**) A transparent acrylic plastic sheet was cut and attached to the top of the main chamber using screws. Unit: mm. R: radius.

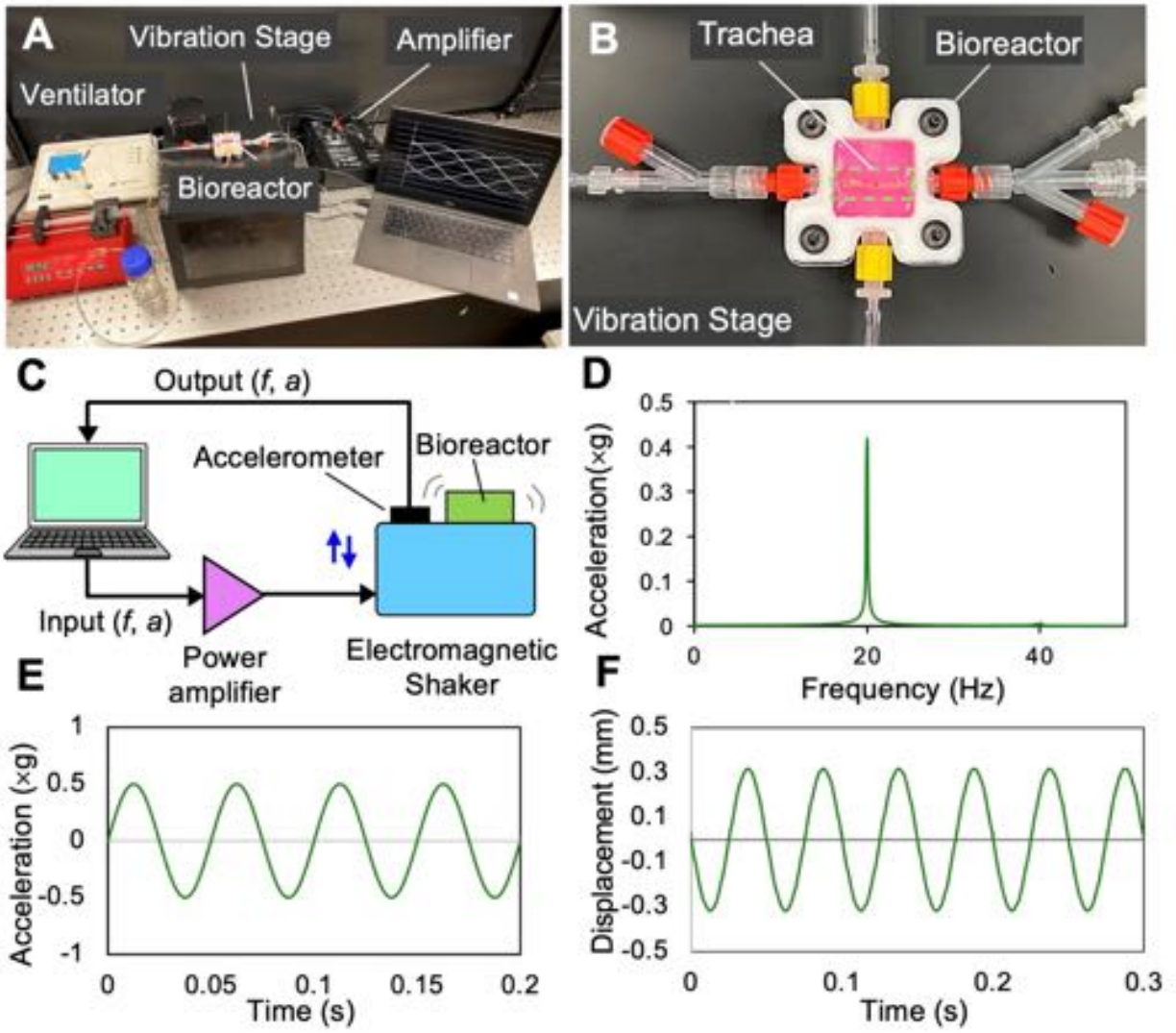


Fig. S2. Mechanical vibration for clearance of detergent-disrupted epithelial cells: (**A**) After cells lysis by SDS detergent solution, the bioreactor containing the trachea was mechanically vibrated using a custom-built electromagnetic shaker. (**B**) Photograph showing the trachea bioreactor placed on the shaker. (**C**) Schematic of the electromagnetic shaker where the oscillation pattern (e.g., *f*: frequency, *a*: amplitude) was controlled by a computerized waveform generator and response was monitored via an accelerometer and a high-frame rate camera. (**D**) Acceleration force measured against frequency showed the maximum force generation at 20 Hz which was the input frequency of the waveform used for tissue vibration. (**E**) Output acceleration force measured against time and (**F**) displacement of the sample stage of the shaker determined using a high-speed camera and subsequent image processing via ImageJ (**Video S1**).

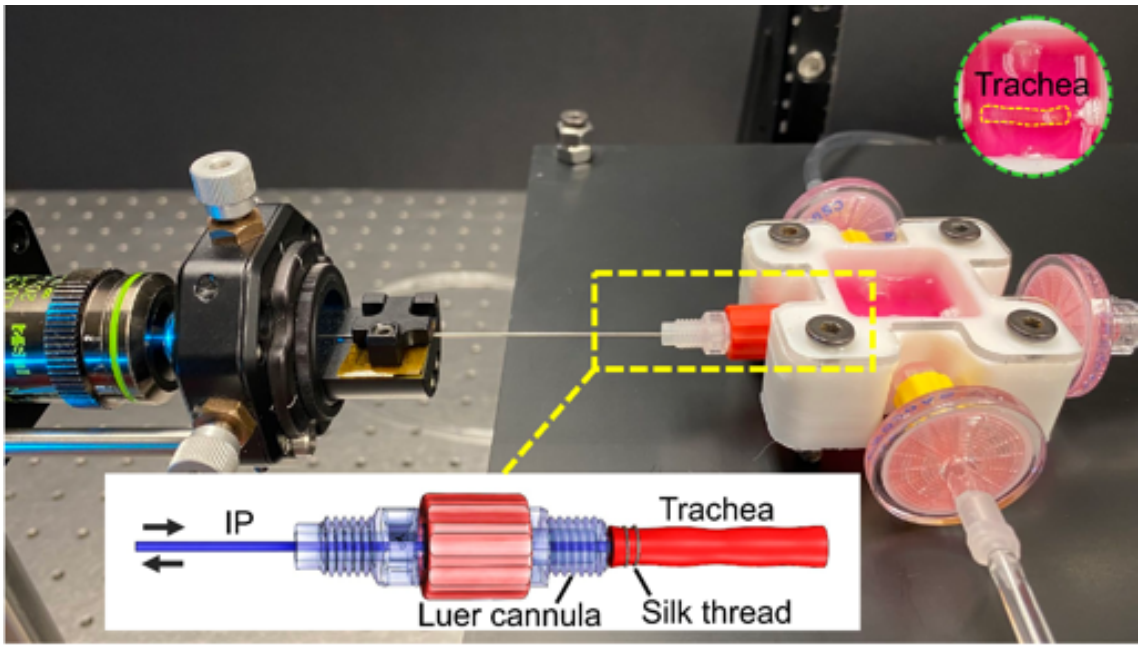


Fig. S3. Introduction of imaging probe into the trachea through the bioreactor connection and luer cannula to visualize the trachea lumen. IP: imaging probe.

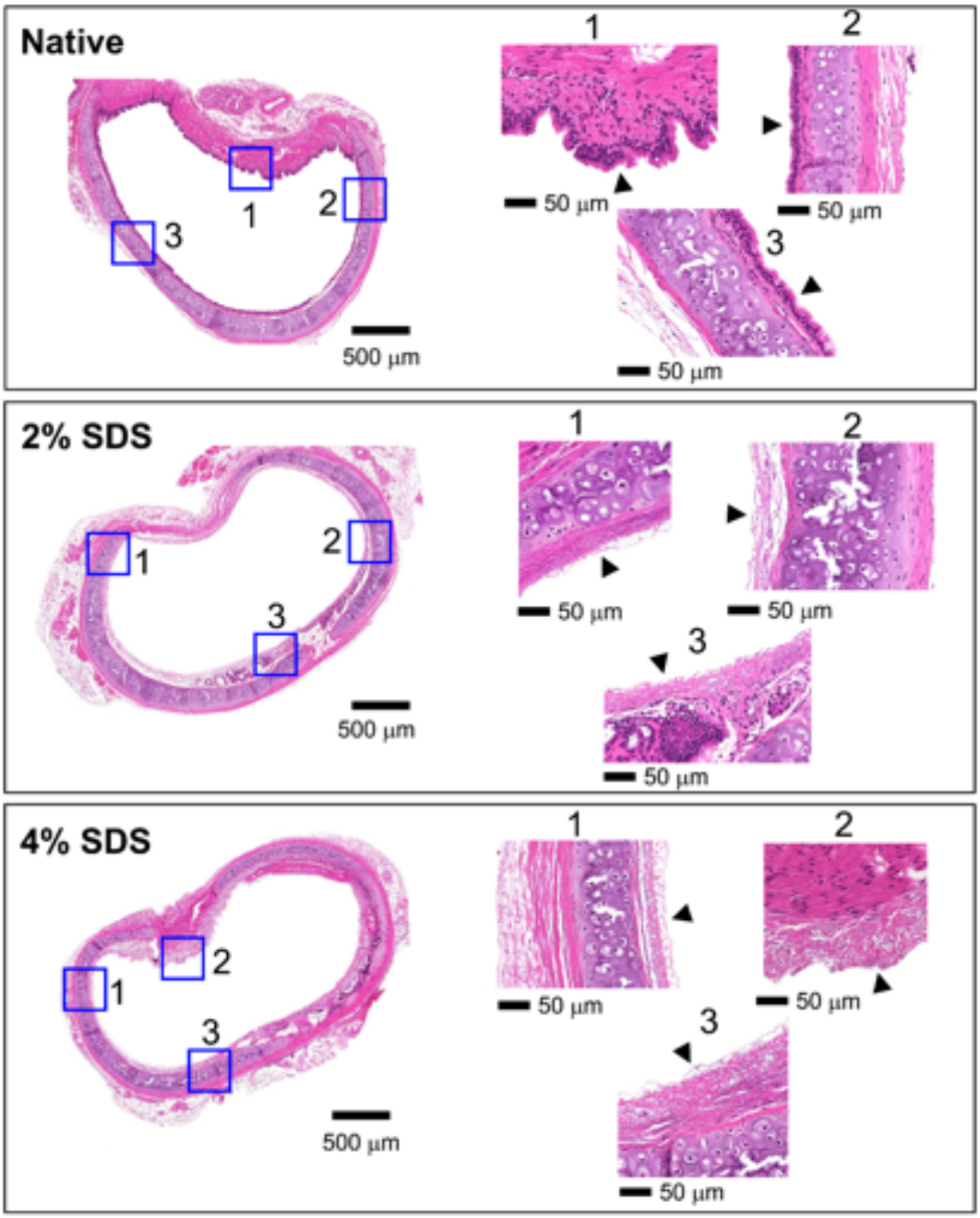


Fig. S4. H&E staining images of the cross-section of native and de-epithelialized rat tracheas. De-epithelialization was achieved by topical deposition of 2% and 4% SDS detergent solution followed by vibration-assisted airway wash. The histology images show the homogeneity of the de-epithelialization across the circumference of the tracheas. Tracheal lumen is indicated by an arrowhead in each image.

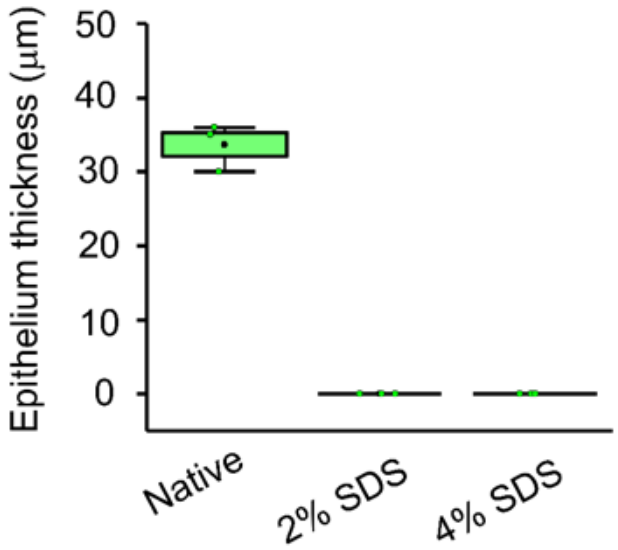


Fig. S5. Quantification of the average epithelium thickness across the circumference of the tracheas shown in Fig. S4. The average epithelium thickness for the native trachea was 33.6 ± 3.2 μm, while the de-epithelialized tracheas treated with 2% and 4% SDS was 0 ± 0 μm.

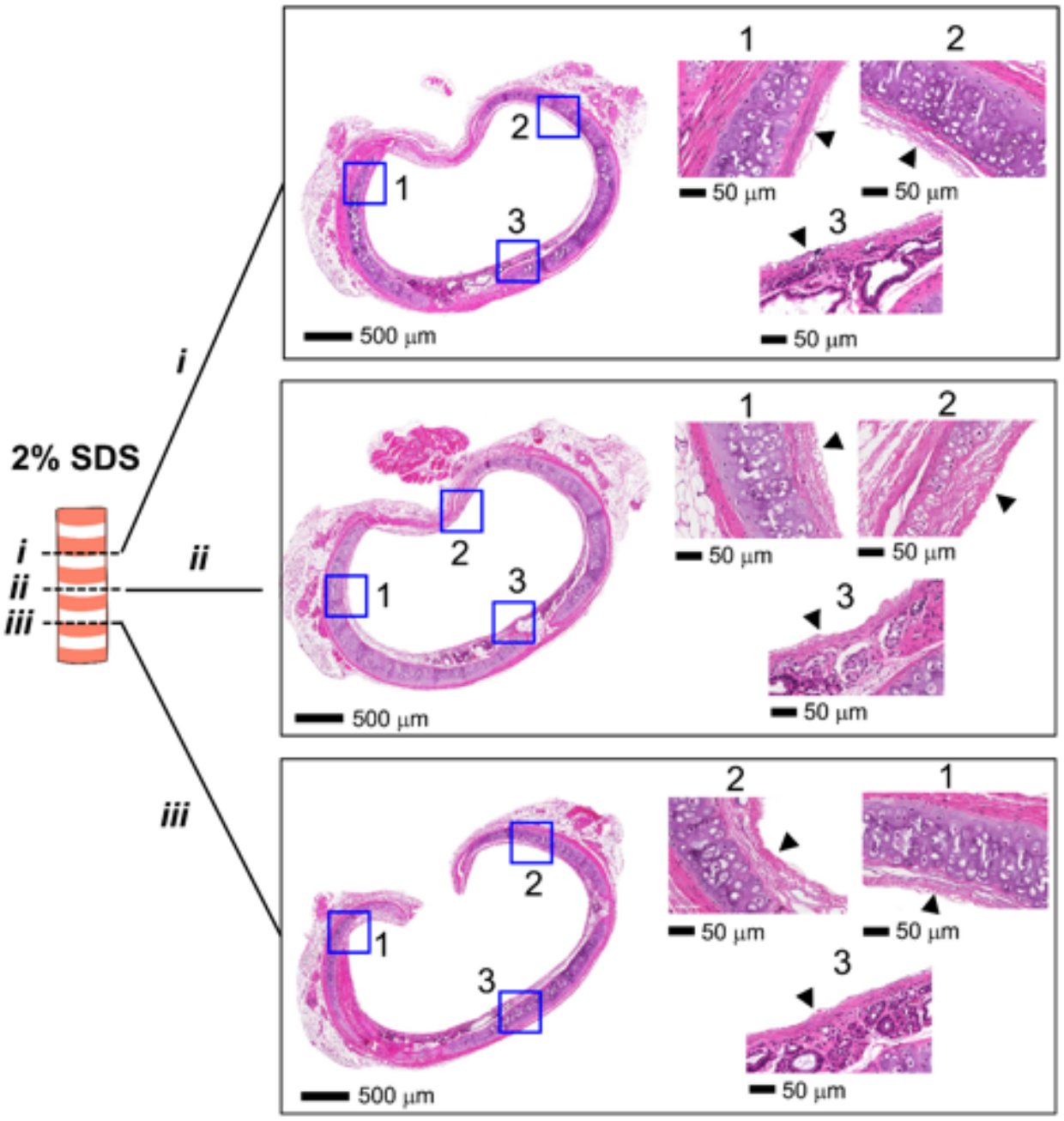


Fig. S6. The homogeneity of de-epithelialization along the length of the trachea that was treated with 2% SDS. The distance between the three evaluated regions (*i*, *ii*, and *iii*) is approximately 0.5 mm. Tracheal lumen is indicated by an arrowhead in each image.

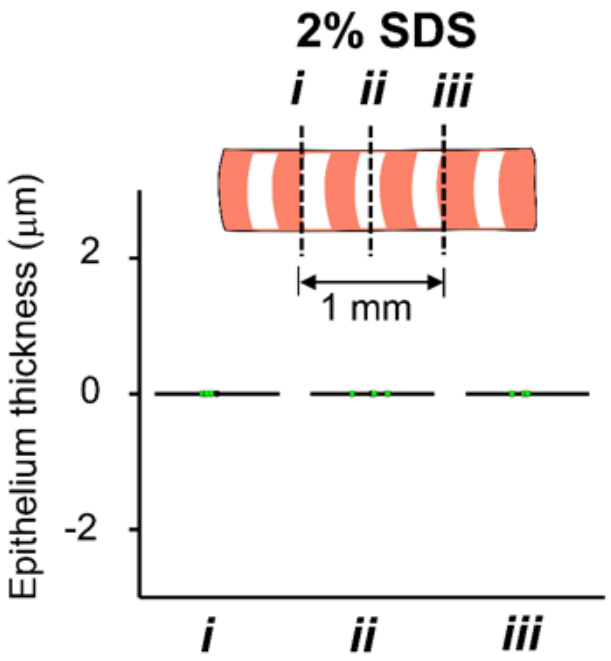


Fig. S7. Quantification of the average epithelium thickness along the length of the trachea shown in Fig. S6. As the average epithelium thickness in all three regions were 0 ± 0 μm, we confirmed complete epithelium removal throughout the entire trachea lumen.

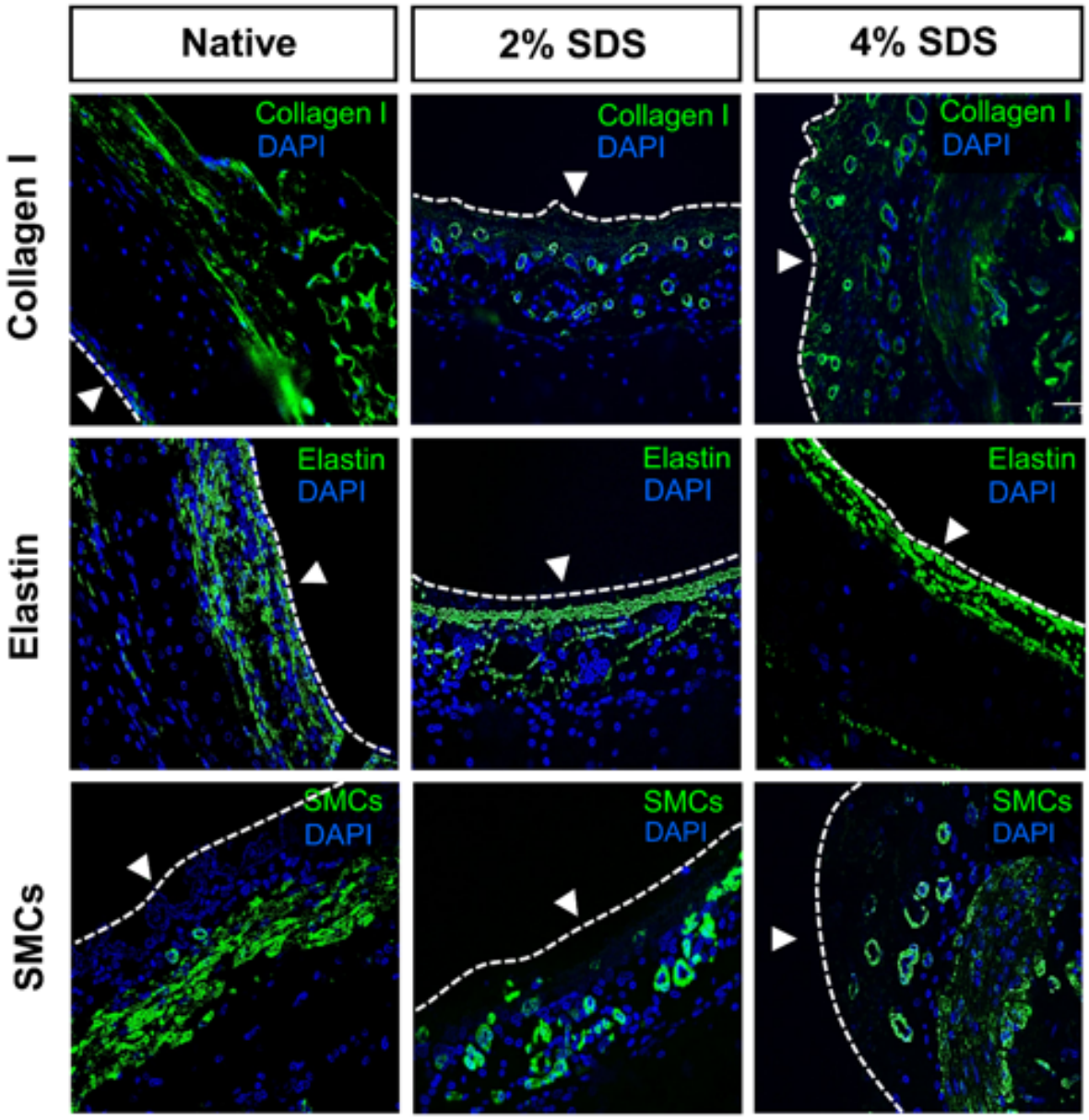


Fig. S8. Immunofluorescence staining of the rat trachea tissues with collagen I, elastin, and smooth muscles (SMCs). Discernible green signals were observed in the tissues that were de-epithelialized with SDS detergent solutions, indicating preservation of the ECM components and structures. Tracheal lumen is indicated by an arrowhead in each image.

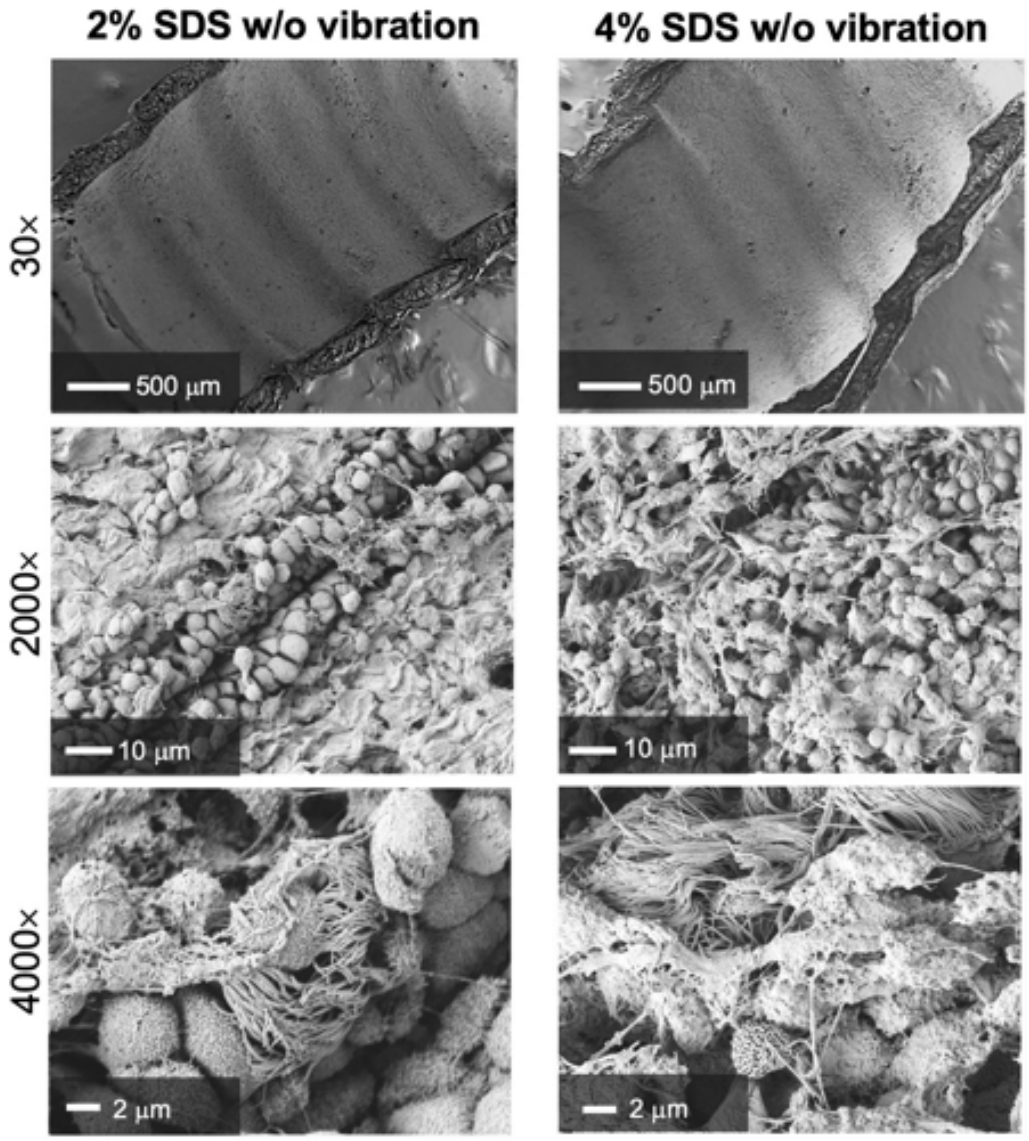


Fig. S9. SEM images showing rat tracheas treated with 2% and 4% SDS solution followed by gentle airway washing in the absence of mechanical vibration. Debris of epithelial cells remained adhered onto the lumen of these tracheas suggested that vibration energy provided to the tissue during de-epithelialization was essential to achieve complete epithelium removal from the airway lumen.

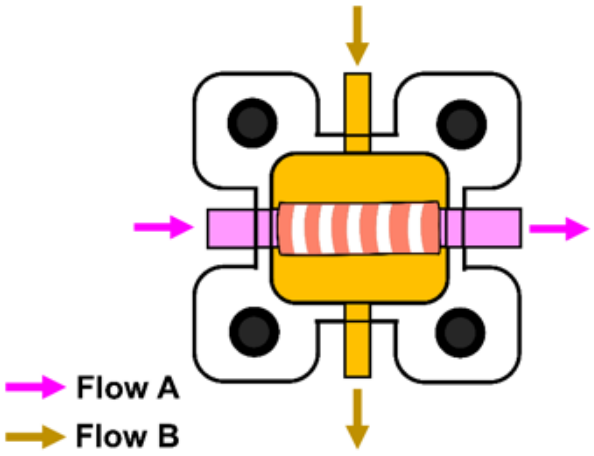


Fig. S10. A schematic showing flow directions of media within the bioreactor. "Flow A" passes through the trachea interior and "Flow B" passes across the exterior of the trachea within the bioreactor chamber.

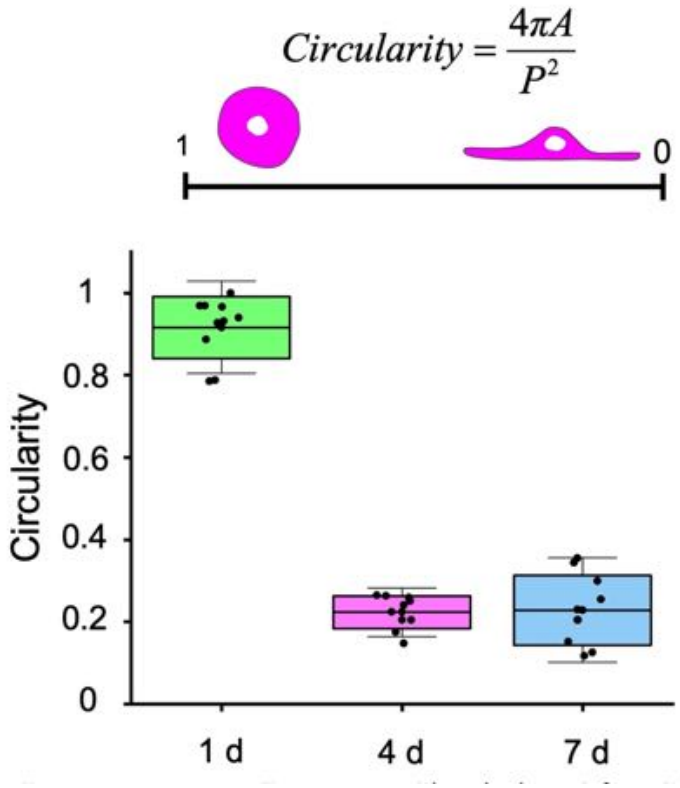


Fig. S11. Circularity of MSCs implanted and cultured on the de-epithelialized tracheas measured at different time intervals (i.e., 1, 4, and 7 days). Cell circularity (range: 0-1) was determined by calculating the ratio of the surface area (A) to the perimeter (P) of the cell. Circularity of the cells decreased substantially to below 0.25 at days 4 and 7, indicating the cells were actively engrafted onto the tissue surface.

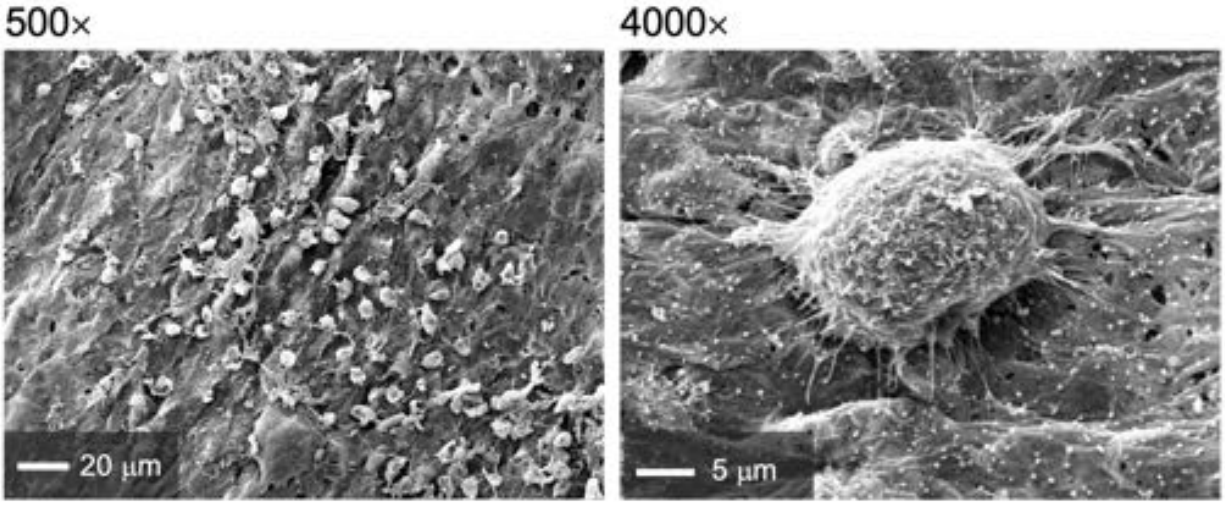


Fig. S12. SEM micrographs of MSCs distributed onto the de-epithelialized rat trachea lumen. Notably, the cells initiated binding onto the tissue surface via multiple protrusions extending from the cell membrane to the surface at day 1.

III. Supplementary Tables

Table S1. Commercial components used to create the bioreactor.

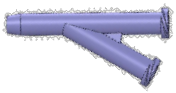
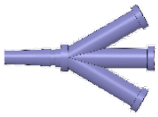
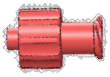
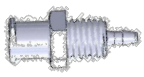
Part	Name of Component	Company	Catalog number
	3-port connector (×1)	World Precision Instruments	14048-20
	4-port connector (×1)	World Precision Instruments	14047-10
	Button head screws (×4)	McMaster-Carr	91255A274
	Female luer to tubing barb (×2)	Cole-Parmer	EW-45508-03
	Female to male luer connector (×2)	Cole-Parmer	ZY-45508-80
	Hex nut (×4)	McMaster-Carr	91813A160
	Luer cannular, female luer bulkhead to hose barb adapter (×2)	Cole-Parmer	EW-45501-30
	Threaded luer adapter (×2)	Cole-Parmer	EW-45513-81
	Tubing	Cole-Parmer	13-200-110

Table S2. List of primary antibodies.

Primary antibodies	Application	Catalog number, Company	Dilution
Rb Anti-Elastin	IF	ab21610, Abcam	1:250
Rb Anti-EpCAM	IF	PA5-19832, ThermoFisher	1:1000
Rb Anti-Collagen I	IF	ab34710, Abcam	1:200
Rb Anti-Alpha Smooth Muscle actin	IF	ab124964, Abcam	1:200
Rb anti-Laminin	IF	PA1-16730, ThermoFisher	1:1000
Rb Anti-CD31	IF	ab28364, Abcam	1:200
Mouse Anti-Rat CD31	IF	MCA1334GA, BioRad	1:100

*IF: immunofluorescence

Table S3. List of secondary antibodies.

Secondary antibodies	Application	Catalog number, Company	Dilution
Donkey Anti-Rabbit IgG (488)	IF	ab150073, Abcam	1:500
Goat anti Mouse IgG (680)	IF	STAR117D680GA, BioRad	1:100
Goat anti-Rabbit IgG (488)	IF	ab97244, Abcam	1:300
Goat anti-Rabbit IgG (555)	IF	ab150078, Abcam	1:500

*IF: immunofluorescence

IV. Legends for Supplementary Videos

Video S1. Movies showing the trachea-loaded bioreactor being mechanically vibrated on our custom-built shaker at 20 Hz of oscillation frequency. The videos were taken at 240 frame per second (fps) and being played at 30 fps.

Video S2. Side view of the sample stage of the shaker being oscillated at 20 Hz. The maximum vertical displacement was ± 0.3 mm with respect to the original position of the stage at still.

Video S3. Internal space of the isolated rat trachea visualized using the GRIN lens imaging probe while illuminating with white light.

Video S4. Internal space of the isolated rat trachea visualized using the GRIN lens imaging probe while illuminating with 488-nm laser. The tracheal lumen was stained with CFSE prior to the imaging and emission light was filtered using a bandpass filter (ET535/50 nm, Chroma®).

V. Supplementary References

[S1] J. Chen, M. Mir, M. R. Pinezich, J. D. O'Neill, B. A. Guenthart, M. Bacchetta, G. Vunjak-Novakovic, S. X. L. Huang and J. Kim, *ACS Biomaterials Science & Engineering*, 2022, **8**, 82-88.