Electronic Supplementary Material (ESI) for Nanoscale.
This journal is © The Royal Society of Chemistry 2023

Supplementary Material

A Safer Framework to Evaluate Characterization Technologies of Exhaled Biologic Materials Using Electrospun Nanofibers

David T. Evans, Dalton J. Nelson, Megan E. Pask, and Frederick R. Haselton*

**Department of Biomedical Engineering, 5824 Stevenson Center, Vanderbilt University, Nashville, TN 37232, USA. Email: rick.haselton@vanderbilt.edu*

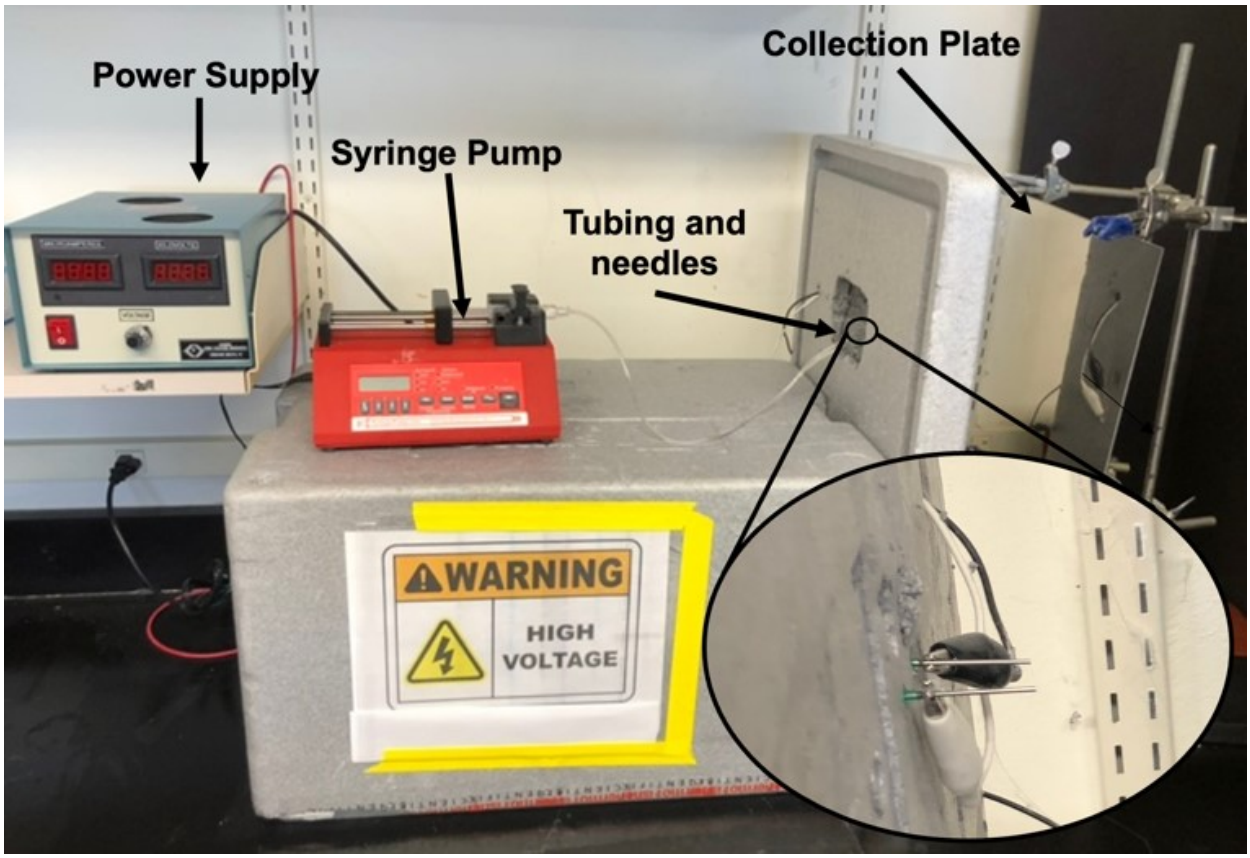

Fig. S1 The electrospinning process set-up used to make the mask inserts. It is designed to form fibrous substrates of PVA by slowly extruding a polymer solution through a needle while applying a high voltage.

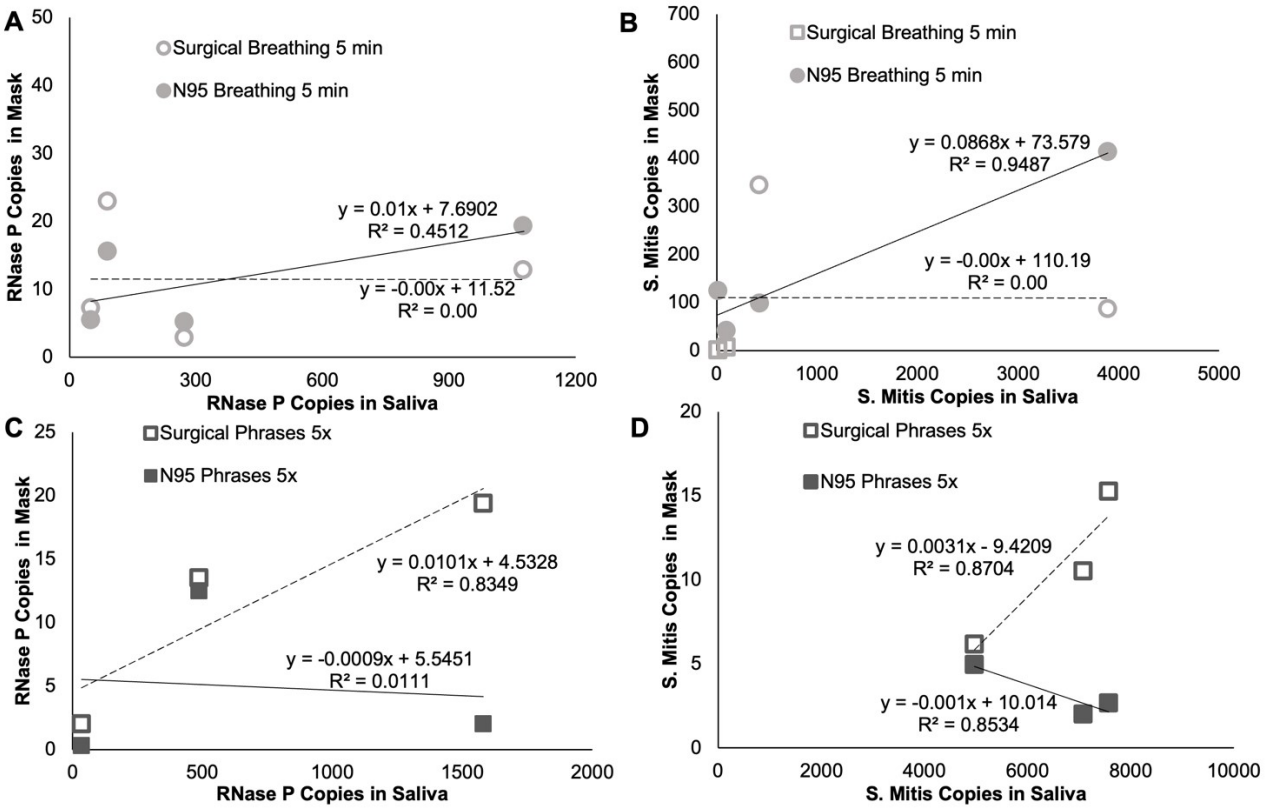

Fig. 2 Comparison of RNase P (A&C) and *S. mitis* (B&D) copies detected in saliva vs mask type. Plots A&B are of the 5 minute breathing samples and plots C&D are of the 5x speaking samples. Each point is a different individual and trend lines are grouped by mask type with the hollow points and dashed lines representing surgical masks and the solid points and lines representing N95 masks.

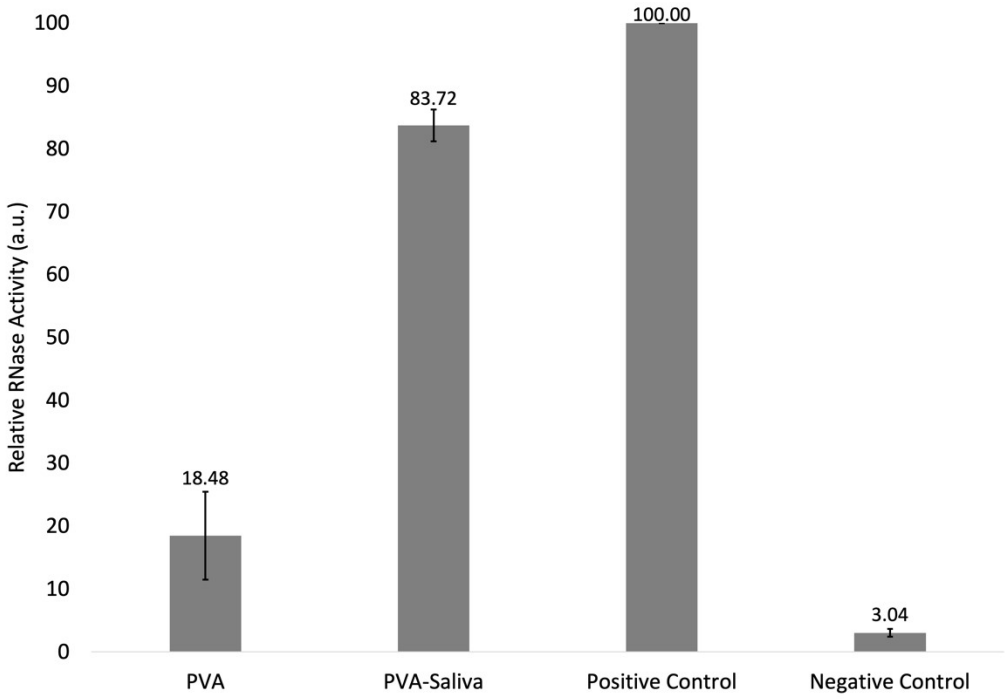

Fig. S3 Relative RNase activity was tested using an RNaseAlert™ kit from Invitrogen (ThermoFisher Cat AM1964). Electrospun PVA inserts were tested with and without 5 μL of saliva spiked on in the same manner as the extraction efficency was. Inserts were dissolved in 100 μL by heating to 60°C and followed by vortexing until dissolved. Negative controls are pure water. Positive controls are RNase A spiked into water. 45 μL of sample was mixed with 5 μL of RNase Alert™ substrate in replicate and incubated at 37°C for 1 hour at which point endpoint fluoresence was measured on a Qiagen Roto-Gene. Replicates were averaged. All samples are N=3 with S.D. error bars. Note 100 is the max readout of the flourometer so the positive samples were likely >100.