### 2. Materials and Methods

#### 2.1 Specimen preparation

Eighteen tails dissected from male Sprague-Dawley cadaver rats (length:  $172 \pm 9$  mm) were used in this study. These rats had served as air controls in inhalation exposure studies, and the inhalation exposure studies all had protocols approved by our Institutions Animal Care and Use Committee. All eighteen tails were collected and put on ice after the rats were euthanized, kept at about 5° C (41° F), and then the tail samples were delivered to our laboratory immediately after. Each tail was wrapped in aluminum foil before being placed on ice to minimize air or water exposure. To preserve the mechanical properties of the tail during testing, all samples were kept around 5° C to ensure that both the onset of rigor mortis and freezing were avoided [1]. Approximately an hour prior to testing, the tail was removed from the ice to warm to room temperature (22° C or 72° F), which was verified using a non-contact surface thermometer. For each of the tails, a 7.5 mm diameter section was measured using a digimatic caliper, and the 7.5-mm-location was marked prior to testing, to ensure the tail diameter (*d*) at the compression location was consistent for all trials.

#### 2.2 Experimental set-up for compression tests

Two experimental testing set-ups were used for the tail compression tests, which are detailed in 2.2.1 and 2.2.2. All compression tests were conducted using the Mach1Motion micromechanical testing system (BioSyntech, Montreal, QC, Canada), which was equipped with a displacement sensor with a resolution of 0.5 µm and a 98 N (10 kg) load cell with a resolution of 4.90 mN (500 mg). The maximum sampling rate of the testing machine was 50 Hz. For all the compression tests, each tail was only used once and then discarded. To ensure representative conditions, the tail was placed in a rigid base platen (Fig. 1), which was identical to the loading

plate used in the new rat tail model [2]. The base platen was fabricated using a 3D printer (polylactic acid), and the testing slot was sloped to match the shape of the rat tails to create a level contact area between the indenter platen and the tails. Additionally, the radius of the testing slot on the base platen was 2-3 times that of the tails, to ensure the tail remained unconfined during compression (no contact with slot walls). The indenter platen used in the current study was a rectangular piece of plexiglass which was substantially more rigid than the rat tail, and it was cut precisely to be 57 mm in length and 25 mm in width (1.5 mm thick). Additionally, the indenter platen had a smooth flat contact surface, simulating the flat vibration platform used in the new rat-tail model (Fig. 1) [2].

#### 2.2.1 Design of compression tests

During the force-deformation testing, the indenter platen was centered over the 7.5 mm diameter mark on the tail, and the indenter platen was applied directly to the top surface of the tail (Fig. 1A & 1B). For the force-deformation testing, a total of nine tails were used. Three tails were tested at each of the three deformation velocities (i.e., 0.15 mm/s, 0.05 mm/s, and 0.015 mm/s), and all tails were compressed to a deformation magnitude of 1.875 mm (25% compression ratio). The deformation time histories are displayed in Fig. 2A to show the differences between the three deformation velocities (Fig. 2A).



Fig. 1. Schematics of the testing setups used. A: front-view schematic of setup for compression testing. B: side-view schematic of setup for compression testing. C: front-view schematic for the compression testing setup used to measure contact width.



Fig. 2. The deformation-time curves. A: deformation-time curves for each deformation velocity. B: deformation-time curve for force-relaxation tests.

For each trial, the pre-conditioning compression process was applied as follows: (1) the indenter platen moved downwards until contacting the tail sample, (2) the tail sample was then compressed until reaching the 1.875 mm deformation, (3) the indenter platen then moved up exactly 1.875 mm, and (4) the indenter then prepared for another compression cycle; for the rest of the compression cycles, the indenter just moved precisely down 1.875 mm and then up 1.875 mm. A total of 10 compression cycles were completed for each trial to guarantee that the tail reached a steady state (Fig. 3). The ninth compression cycle was selected for data analysis as it was consistently the most representative curve of the steady state (Fig 3).

For the 0.15 mm/s deformation velocity, an additional 11<sup>th</sup> compression cycle was done with an 11-minute hold executed immediately after completion of the deformation, which was used to evaluate the time histories of the force responses during the force-relaxation of the tails. The deformation-time relationship for the 11-minute hold used for the force relaxation testing is displayed in Fig. 2B.

## 2.2.2 Contact width measurement

For the contact width measurement, an additional nine tails were used. Three tails were tested at each of the deformation magnitudes (i.e., 0.75 mm, 1.35 mm, and 1.875 mm); all tests were performed at a deformation velocity of 0.015 mm/s. To determine the contact width between the tail and the indenter platen during testing, a light coat of baby oil and then baby powder was applied to the tail. A plexiglass piece (1.5 mm thick) identical in size to the indenter platen was then placed on top of the tail (Fig. 1C). The previously marked 7.5-mm-location on the tail was lined up with the center of the plexiglass piece and the center of the plastic indenter

platen, and then the tail was compressed (Fig. 1C). After compression the plexiglass piece was carefully removed. The powder left a detailed outline of the contact width between the tail and the plexiglass piece during compression, which was photographed with a reference scale. All tail contact width data were then evaluated using the images.

# References

[1] Krompecher T, Experimental evaluation of rigor mortis V. effect of various temperatures on the evolution of rigor mortis. Forensic Science International. 1981; 17:19-26.

[2] Dong R, Warren C, Xu X, Wu J, Welcome D, Waugh S, Krajnak K. A Novel Rat Tail Model for Studying Human Finger Vibration Health Effects . Proc IMechE Part H: J Engineering in Medicine, 2023. <u>https://doi.org/10.1177/09544119231181246</u>