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Original Research Article

ORIGINAL RESEARCH ARTICLE

Fabrication and assessment of a bio-inspired synthetic tracheal tissue model for tracheal tube cuff leakage testing

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Abstract

Introduction: Leakage of orogastric secretions past the cuff of a tracheal tube is a contributory factor in ventilator-associated pneumonia. Current bench test methods specified in the International Standard for Anaesthetic and Respiratory Equipment (EN ISO 5361:2023) to test cuff leakage involve using a glass or plastic rigid cylinder model of the trachea. There is a need for more realistic models to inform cuff leakage.

Methods: We used human computerised tomography data and additive manufacturing (3D printing), combined with casting techniques to fabricate a bio-inspired synthetic tracheal model with analogous tissue characteristics. We conducted cuff leakage tests according to EN ISO 5361:2023 and compared results for high-volume low-pressure polyvinyl chloride and polyurethane cuffs between the rigid cylinder trachea with our bio-inspired model.

Results: The tracheal model demonstrated close agreement with published tracheal tissue hardness for cartilaginous and membranous soft tissues. For high-volume low-pressure polyvinyl chloride cuffs the leakage rate was >50% lower in the bio-inspired tracheal model compared with the rigid cylinder model (151 [8] *vs* 261 [11] ml h^{-1}). For high-volume low-pressure polyurethane cuffs, much lower leakage rates were observed than polyvinyl chloride cuffs in both models with leakage rates higher for the bio-inspired trachea model (0.1 [0.2] *vs* 0 [0] ml h^{-1}).

Conclusion: A reproducible tracheal model that incorporates the mechanical properties of the human trachea can be manufactured from segmented CT images and additive manufactured moulds, providing a useful tool to inform future cuff development, leakage testing for industrial applications, and clinical decision-making. There are differences between cuff leakage rates between the bio-inspired model and the rigid cylinder recommended in EN ISO 5361:2023. The bio-inspired model could lead to more accurate and realistic cuff leakage rate testing which would support manufacturers in refining their designs. Clinicians would then be able to choose better tracheal tubes based on the outcomes of this testing.

Keywords: cuff leakage testing; synthetic tissue model; tracheal tissue model; tracheal tube; ventilator associated pneumonia

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Ventilator-associated pneumonia, which occurs in 15–25% of mechanically ventilated patients,[1] increases the duration of mechanical ventilation and length of intensive care stay, and increases mortality by 30%.[2,3] Pulmonary micro-aspiration of orogastric secretions across the cuff of tracheal tubes is one of the main causes of ventilator-associated pneumonia.[4] There is therefore considerable interest in improving tracheal cuff design to ensure adequate seal for prevention of aspiration while minimising cuff mucosal contact pressure, resultant ischaemic injury, and tracheal damage.[5,6]

Bench testing plays a vital role in the design and regulatory compliance of tracheal tubes. The tracheal tube cuff leakage test recommended in the International Standard Anaesthetic and Respiratory Equipment—Tracheal tubes and connectors (EN ISO 5361:2023) involves inserting the tube into a glass or plastic rigid transparent cylinder, inflating the cuff to a predefined pressure and monitoring the leakage rate of fluid past the cuff. Results using this basic cylindrical model suggest that polyvinyl chloride high-volume low-pressure cuffs have high leakage rates compared with other cuff types, that polyurethane cuffs have a much better seal than polyvinyl chloride cuffs, and that leakage rates can vary between tracheal tubes of the same type from different manufacturers.[7–11]

However, it would be beneficial if tracheal tubes could be designed and leakage rates tested using synthetic tracheal models that are more mechanically realistic than rigid smooth cylinders. There is currently no requirement for cuff leakage tests to use synthetic tracheal models that reflect organic tissue morphology and current artificial tracheal models do not mimic the tactile properties of the *in vivo* airway.[12,13] A model could be used by device designers, replacing cadaveric human, *ex vivo* animal, and *in vivo* animal models for the testing and refinement of product design.[14,15] Synthetic, bio-inspired tissue models would offer consistent mechanical responses to soft tissue deformation such as the loading forces exerted during tracheal intubation and cuff inflation. As the same synthetic tissue model could be used repeatedly during device development, results could be readily compared between design iterations. Previous attempts to reproduce the morphology of the human airway have used medical imaging and additive manufacturing to create an analogous trachea, but even the most advanced such models apply only a single hardness in their fabrication based on published biomechanical data.[16] In contrast, *in vivo* human tracheal ring cartilage, vocal cords, and tracheal mucosa membrane vary in hardness by up to an order of magnitude difference.[17–19] Para-tracheal tissues, such as the thyroid and cricoid cartilages, also contribute to whole anatomical response to deformation, but none of these features are considered in current models.

The aim of this study is to fabricate and test a realistic, bio-inspired, tracheal model incorporating the mechanical properties of the human trachea. The study objectives were: first, to characterise the mechanical properties of the model compared with human tissues; second, to assess the performance of the synthetic model according to the EN ISO 5361:2023 test methodology for tracheal tube cuff leakage tests compared with the standard cylindrical tracheal model.

Methods

Biomechanical data

After consultation with the Nottingham University Hospitals NHS Trust Caldicott Guardian, all identifying information (including 'invisible' metadata) was removed from a single non-pathological adult tracheal computerised tomography scan obtained at Nottingham University Hospitals NHS Trust. This comprehensive anonymisation process was informed by the Royal College of Radiologists' guidance on the use of images for teaching, training, and research.[20] After inter-institutional Data Transfer Agreement, computerised tomography data were segmented using dedicated software (Mimics Innovation Suite 23, Materialise, Leuven, Belgium) to separate the anatomy of the airway and its components from the surrounding tissue of the neck and chest. The tracheal mucosa, tracheal cartilages, thyroid cartilage, (tracheal rings), thyroid gland, and cricoid cartilage were segmented as individual anatomical components based on the provided computerised tomography data. Each component is identified based on its optical density relating to its specific morphology (Hounsfield scale). Each individual anatomical component was identified, rendered, and smoothed to remove imaging artifacts created by anatomical motion during data capture, using image processing software (3Matics Materialise, Leuven, Belgium), to create the digital models for additive manufacturing.

Modelling materials

Polydimethylsiloxane gels Platsil© gel 00–30 and Transil© 20 (Neill's Materials, Bury St Edmunds, UK) were adulterated with additives to recreate the anthropomorphic surrogate materials for each region of interest. In the case of the main tracheal mucosa membrane and thyroid gland, Platsil© gel 00–30 was used. For the tracheal cartilages, vocal cords, thyroid cartilage, and cricoid cartilage, Transil© 20 was modified with the addition of polydimethylsiloxane hardener and kaolinite powder to increase the Shore hardness from a baseline 20 Shore A (ShA) to 60 ShA hardness. All materials also contained 2% (by weight) added short-strand (1 mm long), loose polyester fibres.

To characterise each material by hardness (i.e. resistance to indentation), mixtures were degassed at −736 mm Hg for 5 min to evacuate entrapped air and poured into 6 mm thickness gauge moulds for curing.

Model fabrication

The bio-inspired model was fabricated following a methodology previously described.[21] Published values of tracheal cartilages and mucosal soft tissue Young's modulus[17–19] provided data for mechanical characterisation of the materials used to create the final bio-inspired trachea model (Table 1). The Gent equation was used to convert the Young's modulus to Shore hardness scale (for details see Supplementary file). No literature specific to the modulus or hardness of the thyroid gland could be found for comparison so the same value was used for both the tracheal mucosa membrane and the thyroid gland.

Rigid additive manufacturing models of the trachea, thyroid gland, thyroid cartilage, and cricoid cartilage were printed using fused deposition of thermoplastic aliphatic polyester-polylactic acid and smoothed by hand to remove additive manufacturing deposition stratification caused by material layering.

The dimensions and orientation of each additive manufacturing component were checked against the digital model before soft toolings (moulds) were made. Moulds were made of the additive manufacturing components using the

Table 1 Published *ex vivo* biomechanical data.

Component	Young's modulus	Shore hardness	Source
Cartilage	3.2–23 MPa	59.6–91 ShA	Rains, 1992[17]; Sicard, 2018[18]
Tracheal mucosa membrane (TMM) and glandular surrogate material	4–18 kPa	35–40 Sh00	Wang, 2000[19]

polydimethylsiloxane elastomer Transil© 20, (Neill's Materials).

First, the bio-inspired trachea model was cast using a wax-based, thermoplastic polymer clay (Neill's Materials), to create a waste mould that was representative of the internal air volume of the additive manufacturing model, thus preserving the internal morphology and dimensions of the real trachea. Next, coloured and hardened polydimethylsiloxane gel was applied by hand to the waste mould in separate layers. First, the vocal cords were painted on with a fine brush, then the mucosa membrane was poured on in several layers to achieve a smooth even surface layer. Then, using a mould of the outer airway, tracheal cartilage rings only were cast using the hardened polydimethylsiloxane gel that had been thickened with the kaolinite powder sufficiently to stay in place. This occurred while the core (now complete with the vocal cords and mucosa membrane in place) was inserted into the outer moulding containing the tracheal rings, ultimately creating a type of over-moulding with a waste core that could be easily removed later with submersion in hot water. The thickness of each layer was determined and checked against the scan data before the final model assembly. To create the rest of the model, the thyroid gland, thyroid cartilage, and cricoid cartilage were poured into their respective moulds, using the coloured polydimethylsiloxane gel blends previously described. All bio-inspired castings were cured for 12 h. All polydimethylsiloxane moulds were coated in a thin layer of petroleum jelly before casting to prevent bonding.

During final model assembly, anatomical components placements were measured against the digital model, pinned in place, and bonded with thickened polydimethylsiloxane adhesive. Finally, the complete assembly was coated with a thin membrane of red-tinted polydimethylsiloxane gel to mechanically and aesthetically unify the components (Platsil© gel 00–30).

Investment in reproduceable moulds enables us to recreate waste cores and airway anatomy quickly and easily, with the potential to scale up production on demand simply by producing additional copies of the existing moulds.

Characterisation of mechanical properties

Our primary objective was to characterise the mechanical properties of the bio-inspired model compared with human tissues. As we aimed to produce a model for use in cuff leakage testing, we selected material hardness, defined as the resistance to localised deformation, measured using calibrated durometers (Checkline, New York, NY, USA; Shore A for cartilage, and Shore 00 for softer mucosa) as the primary outcome measure of interest. Each durometer was mounted to a desktop test stand (Type 2 RX–OS–4H; Checkline) with a combined load weight of 403 g (Fig. 1). Each specimen was prepared and tested as per the standards set out in ASTM D2240-15(2021), ISO 48-4:201830, and BS/ISO 23529:2023.

Cuff leakage tests

Our second objective was to assess the performance of the bio-inspired model according to the EN ISO 5361:2023 test methodology for tracheal tube cuff leakage tests compared with the standard cylindrical tracheal model. The outcome measure of interest in these experiments, as per EN ISO 5361:2023, is the volume of leaked fluid obtained after 10 min of testing. The leakage of fluid past the cuff of two types of tracheal tube was investigated *in vitro* using the fabricated bio-inspired trachea model and a transparent rigid acrylic cylinder model of the same internal diameter. The tests modify the procedures detailed in Annex F of EN ISO 5361:2023 (for details see Supplementary file). The test was conducted in ambient conditions and the temperature of the distilled water was maintained at 37°C. Tracheal tube cuff pressures were maintained at 27 cm H_2O (as per EN ISO 5361:2023) throughout the experiment, using a three-way connector linking the pilot balloon of the tube to a programmable syringe pump (Aladdin AL-1000, WPI, Hitchin, UK) and digital manometer (Extech HD750, Teledyne FLIR, Wilsonville, OR, USA). Tracheal tubes, the rigid cylinder and bio-inspired tracheal model were conditioned for 20 min in a 37°C water bath, and then dried, before commencing the experiments. Tracheal tube cuffs were inflated and checked by inspection before each test.

Two 7.0 mm internal diameter tracheal tubes were investigated: a high-volume low-pressure polyvinyl chloride tube (P^3 Medical, Bristol, UK) and a high-volume low-pressure polyurethane tube (Halyard Micro, Alpharetta, GA, USA). These tube types were chosen as they are in common clinical use.

The tracheal tube was inserted into the cylinder or the bio-inspired tracheal model. Distilled water was poured into the relevant model and maintained to a depth of 5 cm above the cuff as required in the standard. A beaker, to collect leaked fluid, was placed on an analytical balance (MC1 Laboratory LC4800-P00V, 30906009, Sartorius, Göttingen, Germany) beneath the rig. After 10 min, the amount of leaked fluid was recorded. The leakage tests for each tracheal tube type across the five tracheal tubes were conducted in the same measurement session but not simultaneously. The experimental set up is shown in Fig. 2.

Statistical analysis

Tests were conducted on five high-volume low-pressure polyvinyl chloride cuff tubes and five high-volume low-pressure polyurethane cuff tubes with the test for each single tube repeated 10 times in both cylinder and bio-inspired trachea models. A total of 200 leakage tests were therefore performed. The size of this feasibility sample is in keeping with previously published experiments relating to tracheal cuff leakage.[8]

A mixed effects model was used to compare the results of the bio-inspired model with the cylinder model for the

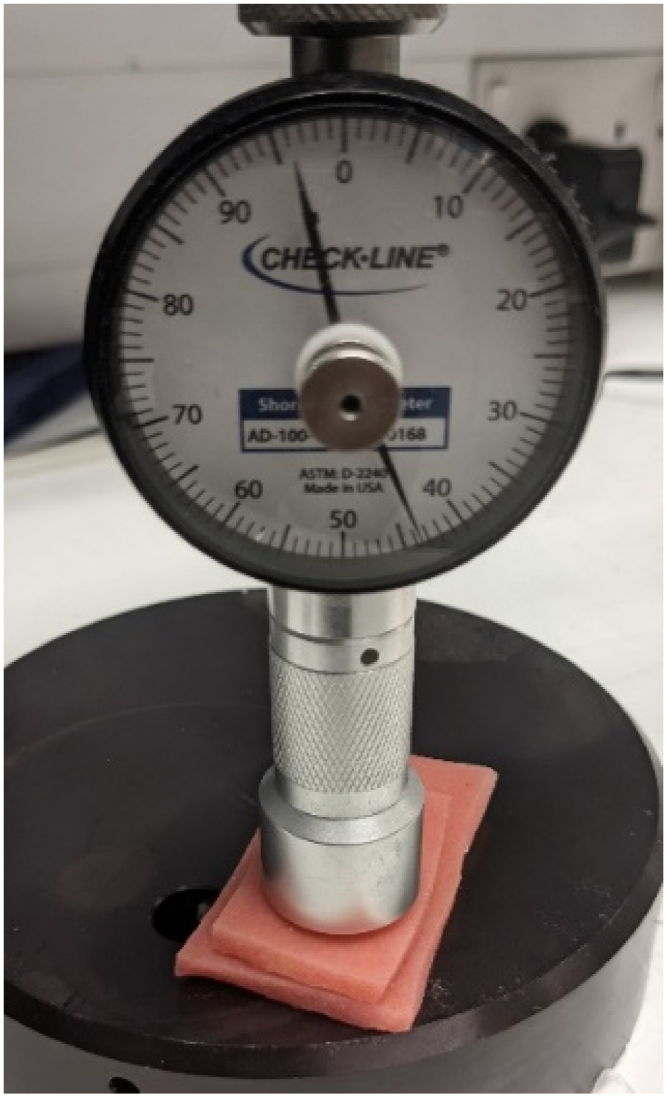

Fig 1. Hardness testing of surrogate tracheal material with stand mounted Shore 00 calibrated durometer.

polyvinyl chloride cuff tracheal tube. The tracheal models were set as fixed effects while the tracheal tubes and the leakage tests were crossed random effects. For the polyurethane cuff tracheal tubes, there was no measurable leakage from the cylinder model and because of this, a formal statistical comparison between the two models was not feasible.

Results

Fabrication and mechanical characterisation of model tissues

The final model is shown in Fig. 3. The materials used in the fabrication of the bio-inspired model displayed good agreement with *ex vivo* tissue characteristics previously described in the literature (Table 1). Mean (95% confidence interval) tracheal mucosal membrane and glandular material hardness was 41.8 (40.4–43.2) Sh00. Cartilage material hardness was 57.2 (56.5–58.7) ShA.

Cuff leakage tests

Leakage rates from the five high-volume low-pressure polyvinyl chloride cuffed tubes are shown in Fig. 4. Mean (standard deviation) leakage rates around high-volume low-pressure polyvinyl chloride cuffs were significantly lower in the bio-inspired tracheal model than in the rigid cylinder model (151 [8] *vs* 261 [11] ml h^{-1}; $P<0.0005$). This represents a 56–60% reduction in observed leakage rates for polyvinyl chloride cuffs when using the bio-inspired tracheal model. The mixed effects analysis shows that the cylinder model is expected to produce 111 ml more leakage than the bio-inspired model when the effects of the tracheal tubes and the leakage tests are controlled for. This difference is significant ($P<0.0005$). The

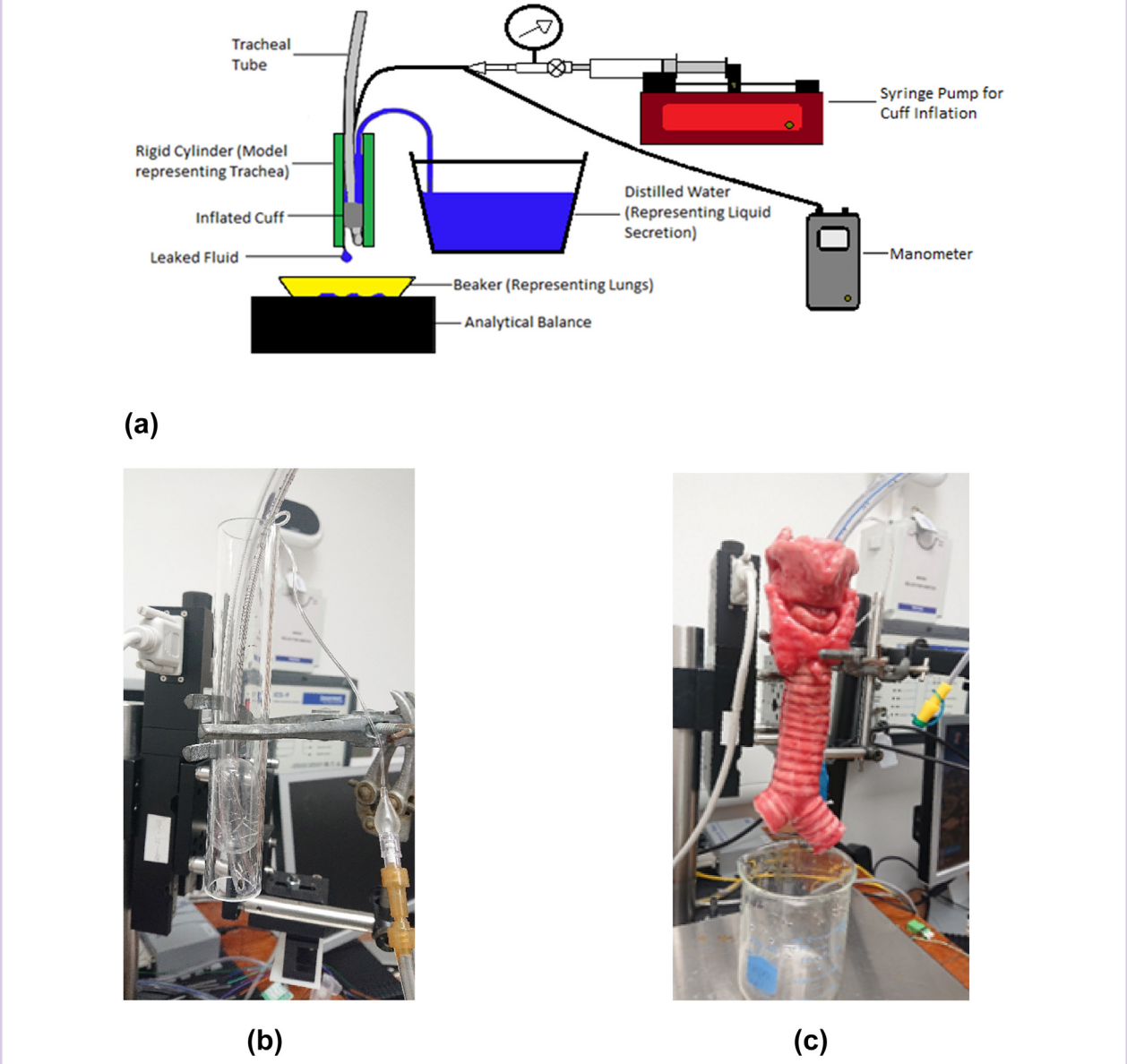

Fig 2. Cuff leakage test experimental set up. (a) System diagram (adapted from EN ISO 5361:2023). (b) Traditional rigid cylinder model. (c) Bio-inspired tracheal model.

tracheal tubes accounted for 91% of the variation in the experiment (for details see Supplementary file).

Leakage rates from the five high-volume low-pressure polyurethane cuffed tracheal tubes are shown in Fig. 5. The leakage rates were much lower for the polyurethane cuffed tubes. Three of the five tracheal tubes produced a measurable amount of leakage for at least six of the 10 tests performed in the bio-inspired tracheal model, with a maximum leakage value of 0.36 ml. There was no measurable leakage around the five high-volume low-pressure polyurethane cuffs when the cylinder model was used (0.1 [0.2] *vs* 0 [0] ml h^{-1}). This demonstrates a difference in the performance of the cylinder and bio-inspired tracheal models with the polyurethane cuff tracheal tube (for details see Supplementary file).

Discussion

A novel bio-inspired tracheal model was created and applied to cuff leakage testing. In this laboratory study we used anonymised radiological data to fabricate and test a novel bio-inspired tracheal model. We demonstrated fundamental model mechanical characteristics, specifically tissue hardness, of both cartilage and mucosal membrane, to be in close agreement with published *ex vivo* data. Data were used from *ex vivo* sources because of the destructive nature of the mechanical testing required for obtaining *in vivo* data. We assessed this model in tracheal tube cuff leakage tests compared with the traditional cylindrical model and showed significant differences in leakage results between the models

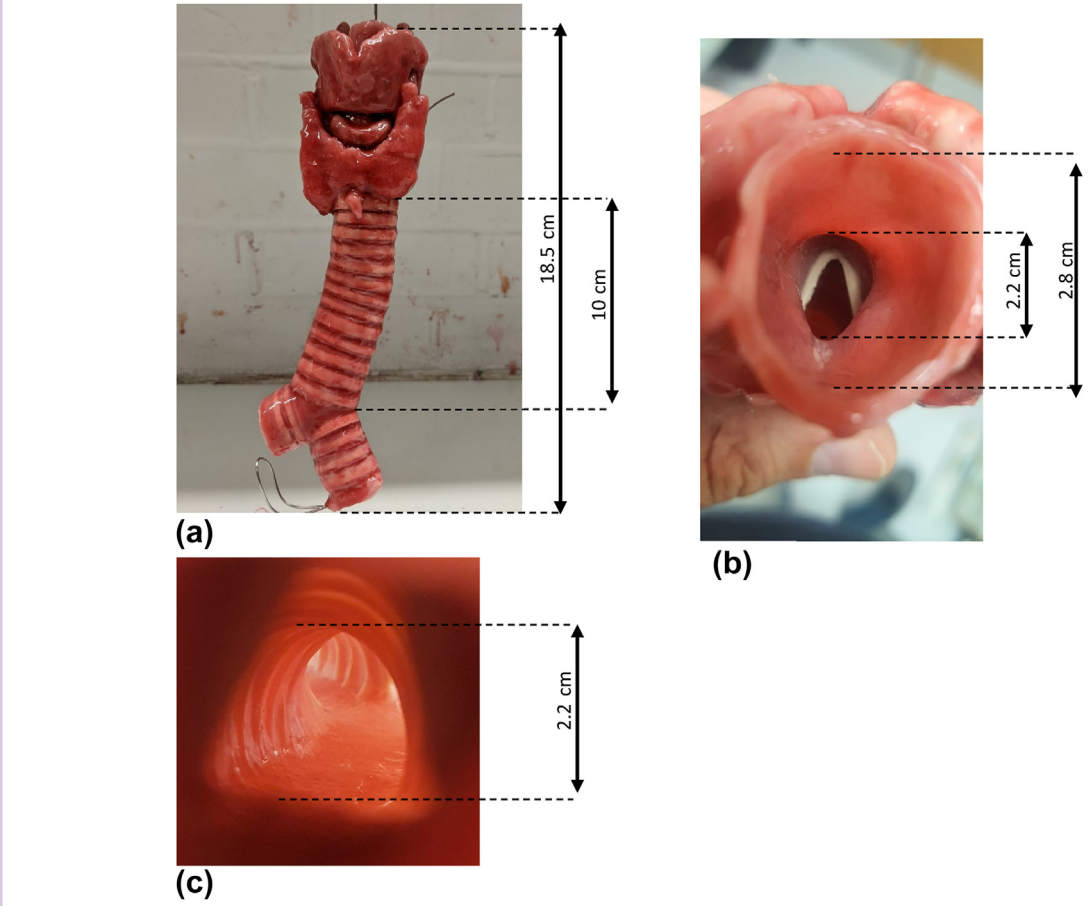

Fig 3. Bio-inspired tracheal model. (a) External view of anterior aspect. (b) External view from top. (c) Internal view of tracheal lumen and mucosa.

with both high-volume low-pressure polyvinyl chloride and polyurethane cuffed tracheal tubes.

High-volume low-pressure cuffs are in common clinical use because they pose a lower risk of tracheal injury compared with low-volume high pressure cuffs.[22] Our finding of leakage across high-volume low-pressure cuffs using the bio-inspired tracheal model supports the hypothesis that the high volume results in folds in the cuff which can serve as channels for secretions into the lungs.[23] We found much higher bio-inspired tracheal model leakage rates with polyvinyl chloride compared with polyurethane cuffs, which is in keeping with previously published *in vitro* findings of Dullenkopf and colleagues[8] and Koka and Philip.[22]. The thin structure of the polyurethane cuff (7–10 μm) forms a better seal with the tracheal mucosa compared with the much thicker polyvinyl chloride cuff (50–70 μm) and reduces leakage.[9] There is no consensus on the minimum clinically important volume of tracheal leakage that constitutes microaspiration. We are therefore unable to state whether the different leakage rates observed past polyurethane cuff between bio-inspired and traditional cylindrical models (0.12 [0.18] *vs* 0 [0] ml h^{-1}), are clinically relevant. Given the mean duration between tracheal intubation and ventilator-associated pneumonia has been reported to be 3 days, this equates to a leakage difference of 8.6 ml between models over this clinically relevant timeframe.[4]

Polyvinyl chloride cuff leakage tests showed a 56–60% difference in leakage rates between the cylinder model and the bio-inspired model for each of the five tracheal tubes of this type. We hypothesise that high-volume low-pressure cuff folds are less of a problem when the luminal walls are flexible, as in the bio-inspired model, because the thick folds fit better with the corrugated shape of the tracheal cartilage rings. In the inflexible cylinder model, the polyvinyl chloride folds become more pronounced and, because of the thickness of the polyvinyl chloride cuff, gaps are created which act as channels through which fluid can leak. Similarly, low leakage rates for the polyurethane cuff in the bio-inspired model may be caused by interaction with the cartilage rings to form thin polyurethane folds and consequent failure to make a perfect seal.[24–26] There was no measurable leakage for the polyurethane cuff in the cylinder model. We believe that the thin structure of the polyurethane cuff placed in the smooth uniform cylinder model forms good contact with the cylinder surface and leaves no path for leakage. In the case of the bio-inspired model there is a variation of leakage rates because of the non-uniform properties of the model creating kinks and folds in the cuff. The size of the thin folds formed on the

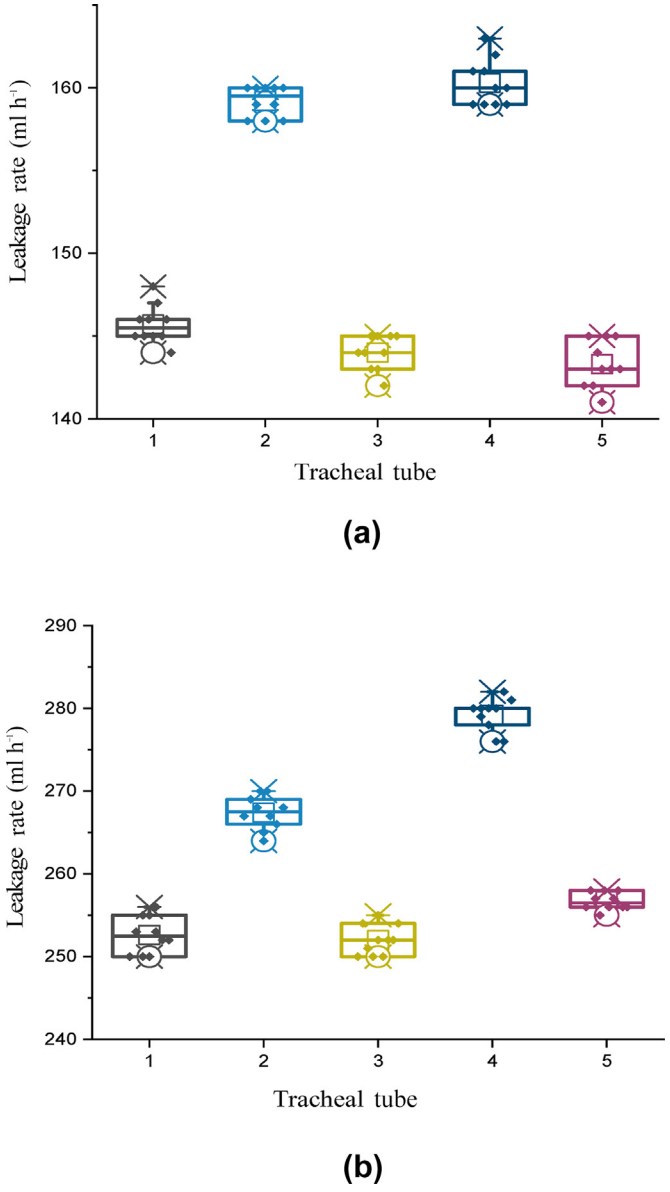

Fig 4. Box plot showing leakage rates for polyvinyl chloride high-volume low-pressure cuffed tracheal tubes. Horizontal line, median; box, inter-quartile range; whiskers, range; square, mean; diamond dots, data points. (a) Bio-inspired model; (b) cylinder model.

polyurethane cuff walls is dependent on the placement of the tracheal tube. There is a greater chance of leakage when the folds form on the corrugated cartilage region compared with the continuous muscle region of the model. We hypothesise that this is the reason behind the variation in the leakage results in the bio-inspired model (Fig. 5a) and that the results of the polyurethane cuffed tube are a better reflection of expected results in surgical settings. Each set of the five tracheal tubes used had similar leakage rates with slight variations. Although the leakage tests were conducted in the same measurement session, with the same ambient conditions and conditioned in a water bath of the same temperature, the tests not being conducted simultaneously would have an impact on the results. We believe that this is the reason for the variability of the leakage results across the tracheal tubes.

This work has several important limitations. First, we did not perform comparative leakage tests on living subjects. Therefore, we are unable to state whether the bio-inspired model leakage performance is more closely aligned to living tissues compared with the traditional cylindrical model. This requires additional *in vivo* comparative testing, which was not within the scope or resource of this work, would be extremely challenging in practice, and be unsuitable for industrial design, testing, and commercial validation purposes. Second, our bio-inspired model is derived from radiological data from one living adult subject and therefore does not incorporate the

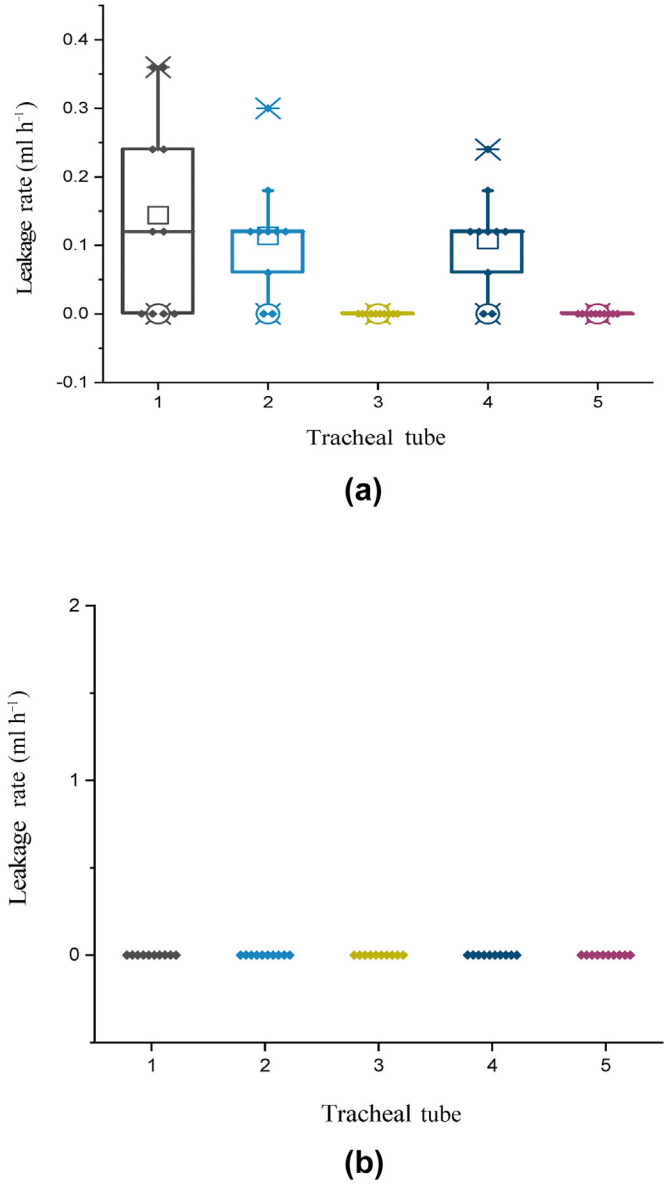

Fig 5. Leakage rates for polyurethane high-volume low-pressure cuffed tracheal tubes. (a) Box plot for bio-inspired model, horizontal line, median; box, inter-quartile range; whiskers, range; square, mean; diamond dots, data points. (b) For the cylinder model no box plot is shown as there is no measurable leakage.

variable anthropometry expected in human populations. Although our radiological data were extracted from a non-pathological trachea, further work could usefully identify the anthropometric range of adult tracheal properties to inform future bio-models. Third, given that the data we used were from *ex vivo* sources, the temperature for hardness testing differed from that of the *in vivo* tracheal tissues by around 15°C. As temperature influences soft tissue compliance, these values might be slightly higher than those of the living (warmer) trachea.

The bio-inspired trachea model has the potential to impact on tracheal tube design and bench testing. Medical intubation device designers rely on human cadavers or animal specimens to test and refine products.[14,15] The process of device design requires many months of work to achieve an effective solution to specific problems, but during this period many different specimens may be needed to iteratively test prototypes. Because different specimens are used at multiple intervals in device development, this introduces uncontrollable variables that can confound test results. This causes difficulties for medical device designers, who require consistent performance from test specimens to reliably test and refine their designs effectively. The bio-inspired trachea model also has the potential to change the recommended testing methods

used in the International Standard for Anaesthetic and Respiratory Equipment (EN ISO 5361:2023) as it introduces considerable variation into the cuff performance results, and in the absence of a gold standard, it supports an argument for a reproducible standardised model with good tissue fidelity. If leakage rates were similar irrespective of the model, then continued use of rigid cylinders would be justified. The model can be readily introduced as, once computerised tomography images have been segmented and moulds 3D-printed, it can be manufactured rapidly, reproducibly, and at low cost to provide a range of stable, anthropometric tracheal models. These features mean that there is potential for widespread use in industry and academia.

Authors' contributions

Design and conduct cuff leakage experiments: TA, RC
First draft of paper: TA
Data analysis: TA, RA, DWH, AMN, SK, BRHG, SPM
Design and of synthetic tissue model: RA, DWH, SE, AS
Construct of synthetic tissue model: RA, AS
Writing model fabrication sections of paper: RA
Writing clinically relevant sections: DWH, AMN
Design of experiments: AMN, SK, BRHG, SPM
Initiation of research writing final versions of paper: SPM

Declarations of interest

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Data access statement

All cuff leakage data created during this research are openly available from the University of Nottingham data repository at https://doi.org/10.17639/nott.7426. Due to ethical concerns, CT scan data cannot be made openly available.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bjao.2024.100290.

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