Respiration and the Airway

Tracheal tube biofilm removal through a novel closed-suctioning system: an experimental study

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Abstract

Background: Tracheal tube biofilm develops during mechanical ventilation. We compared a novel closed-suctioning system vs standard closed-suctioning system in the prevention of tracheal tube biofilm.

Methods: Eighteen pigs, on mechanical ventilation for 76 h, with P. aeruginosa pneumonia were randomized to be tracheally suctioned via the KIMVENT* closed-suctioning system (control group) or a novel closed-suctioning system (treatment group), designed to remove tracheal tube biofilm through saline jets and an inflatable balloon. Upon autopsy, two tracheal tube hemi-sections were dissected for confocal and scanning electron microscopy. Biofilm area, maximal and minimal thickness were computed. Biofilm stage was assessed.

Results: Sixteen animals were included in the final analysis. In the treatment and control group, the mean (SD) pulmonary burden was 3.34 (1.28) and 4.17 (1.09) log cfu gr−1, respectively (P=0.18). Tracheal tube P. aeruginosa colonization was 5.6 (4.9–6.3) and 6.2 (5.6–6.9) cfu ml−1 (median and interquartile range) in the treatment and control group, respectively (P=0.23). In the treatment group, median biofilm area was 3.65 (3.22–4.21) log10 μm2 compared with 4.49 (4.27–4.52) log10 μm2 in the control group (P=0.031). In the treatment and control groups, the maximal biofilm thickness was 48.3 (26.7–71.2) μm (median and interquartile range) and 88.8 (43.8–125.7) μm, respectively. The minimal thickness in the treatment and control group was 0.6 (0–4.0) μm and 23.7 (5.3–27.8) μm (P=0.040) (P=0.017). Earlier stages of biofilm development were found in the treatment group (P<0.001).

Conclusions: The novel CSS reduces biofilm accumulation within the tracheal tube. A clinical trial is required to confirm these findings and the impact on major outcomes.

Key words: biofilms; catheters; intubation intratracheal; pneumonia

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Tracheal tube (TT) biofilms are multifaceted microbial communities adherent to the TT plastic surface, and embedded within an exopolysaccharide matrix. TT biofilm rapidly develops during the course of mechanical ventilation (MV), and it is consistently found at extubation. Importantly, retained respiratory secretions often overlay TT biofilm and form a miscellaneous biostructure within the tube.

Tracheally intubated patients may inhale pathogens dislodged from the TT biofilm, and antibiotics exert marginal bactericidal activity against sessile pathogens. This may lead to the development of ventilator-associated pneumonia (VAP). Especially when biofilm is allowed to mature to its final stages of development. Moreover, retained secretions and biofilm narrow the TT internal lumen, leading to an increase in airflow resistance and the patient’s work of breathing. Thus, a few devices have been developed to hinder TT biofilm formation. TTs coated with silver deter the initial adherence of pathogens on the TT surface. In laboratory and clinical trials silver-coated TTs have shown delayed biofilm formation. Yet, the efficacy of coated TTs seems to weaken over time, as colonized mucus builds up within the TT. Dedicated apparatus, such as the Mucus Shaver, or similar devices, have been used to remove secretions and biofilm from the TT. Nevertheless, all of these devices are used after TT suctioning and require disconnection from the ventilator circuit.

A novel closed suctioning system (CSS) has been developed to dislodge TT biofilm, through high-pressure jets of saline and an inflatable balloon, and to aspirate respiratory secretions and biofilm debris. Here we report the results of a randomized laboratory study, in mechanically ventilated pigs with Pseudomonas aeruginosa pneumonia to compare the efficacy and safety of the novel device with standard CSS, in the prevention of TT biofilm formation.

**Methods**

The Institutional Ethics Committee approved the protocol. Animals were managed according to the National Institutes of Health guidelines for the use and care of animals. Full methodological details are provided in the online supplement and relevant aspects of the ARRIVE guidelines were adhered to.

**Animal preparation and handlings**

Eighteen Large White–Landrace female pigs (weight, 32.6 (2.9) kg) were induced, intubated with a 7.5 internal diameter (ID) TT (Hi-Lo EVAC, Coviden, Boulder, CO) and connected to a mechanical ventilator (SERVO-I, Maquet, NJ). Sedation and analgesia were maintained as previously reported. Internal TT cuff pressure was maintained through a mechanical device. Pigs were ventilated in volume-control, and PEEP and respiratory rate adjusted to maintain arterial partial pressures of oxygen and carbon dioxide within the physiological range. Inspiratory gases were conditioned through a heated humidifier (Conchatherm III, Hudson RCI, Temecula, CA). Ceftriaxone was administered throughout the study to prevent endogenous colonization. We surgically cannulated the femoral artery for systemic arterial pressure monitoring and collection of blood samples. Additionally, a Foley catheter was introduced into the bladder, through mini-pelvectomy. After surgical preparation, pigs were placed in lateral Trendelenburg (−5°) position. Every 6 h, the animal was turned from one lateral side to the other.

**Randomization**

Pigs were randomized as described in the online supplement into the following groups:

**CONTROL GROUP:** Tracheal suctioning was performed using a 12-Fr standard CSS (KIMVENT* Closed Suction Systems, Kimberly Clark, Irving, TX), as clinically recommended. **TREATMENT GROUP:** The novel 12-Fr CSS (Airway Medix Closed Suction System, Biovo Technologies, Israel) was used as illustrated in Fig. 1. Before the beginning of the study, all investigators underwent training to learn how to operate the device.

In both groups, tracheal suctioning was performed every 6 h, or when clinically indicated by visible secretions within the TT;
pathological sounds; increased peak inspiratory pressure; saw-tooth pattern on the expiratory flow waveform.\textsuperscript{23} Quantity of aspirated secretions was estimated with a semi-objective scale from 0 to 4 (none, few, mild, moderate, abundant); additionally, the quality of mucus (normal or purulent) was recorded.\textsuperscript{24} In the treatment group, quantity of aspirated secretions was assessed before the activation of saline jets. Negative pressure was set to 150 mm Hg. In both groups, we aimed at performing the suctioning procedure in <15-sec.\textsuperscript{23} The final suctioning was performed three h before the pigs were killed. Full details are provided in the online supplement.

**Model of \textit{P. aeruginosa} pneumonia**

As previously reported,\textsuperscript{25} after surgical preparation and stabilization, 15 ml of a 10^8 cfu ml\textsuperscript{-1} suspension of ceftriaxone-resistant \textit{Pseudomonas aeruginosa} ATCC 27853 were inoculated into each lobe using a bronchoscope.

**Respiratory measurements**

Every 24 h, one hour after tracheal suctioning, we recorded airway pressure and respiratory flow rates to compute airflow resistance using standard formulae.\textsuperscript{26} Additionally, every 24 h, gas exchange was assessed.

**Autopsy and microbiological studies**

Animals were killed after 76 h of invasive MV. Upon autopsy, animals were kept in the reverse-Trendelenburg position to avoid post-mortem displacement of mucus into the TT. We took a tissue sample from each lobe of the lung for quantitative culture.\textsuperscript{21} \textit{P. aeruginosa} pneumonia was microbiologically confirmed according to lobar bacterial culture $\geq 3$ log cfu gr\textsuperscript{-1}.\textsuperscript{27, 28}

**TT microbiological and microscopy studies**

Upon extubation, the TT was sealed with clamps. The external surface was cleaned with sterile gauzes and decontaminated. Then, the TT was longitudinally cut open and pictures of the entire TT length were taken. These pictures were analysed and the gross appearance of the TT lumen scored, by an operator blinded to the treatment allocation, as follows: 0, no mucus; 1, mucus covering $<10\%$; 2, 10–25\%; 3, 25–50\% and 4, $>50\%$ of the TT length.\textsuperscript{3} The TT internal surface was gently rinsed with saline to remove non-adherent mucus. As reported in the on-line Supplementary data, Fig. 1, three sections of the dependent half – based on the last pig positioning before euthanasia – were dissected to quantify Gram-negative aerobic pathogens and analyse TT biofilm through scanning electron microscopy (SEM)\textsuperscript{29} and confocal scanning electron microscopy (CSLM).\textsuperscript{30} CSLM axial images of representative biofilm accumulations were recorded and analysed via dedicated

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**Fig 2** Gross-appearance of the tracheal tube internal tracheal tube surface. The gross appearance of the tube was scored as follows: 0, no mucus; 1, mucus covering $<10\%$; 2, 10–25\%; 3, 25–50\% and 4, $>50\%$ of the tube length. Of note, in pig numbers 81, 105, 108 and 112, (control group) more than 50% of the tracheal tube internal surface length was covered by mucus. In pig numbers 95 and 106, (treatment group), no mucus was found on the tracheal tube internal lumen. Only 7 out of 8 control tracheal tubes are reported, because of accidental loss of collected pictures.
software (ImageJ, NIH, Bethesda, MD, USA). Biofilm area, maximal and minimal thickness were computed. Biofilm stage was assessed through SEM micrographs analysis as previously reported. It is important to emphasize that on the TT surface, biofilm structure is highly heterogeneous and characterized by areas with significant accrual, and regions with marginal biofilm presence. We planned to take one CSLM and SEM picture of each representative biofilm accumulation on the TT surface to fully characterize biofilm. Data from multiple CSLM pictures from each pig were averaged for the final analysis. During microscopy analyses, investigators were blinded to treatment allocation.

Safety

Upon suctioning, ventilatory and haemodynamic parameters were monitored and any associated complication (sustained oxygen desaturation, bronchoconstriction, sustained hypertension/hypotension or cardiac dysrhythmias) recorded. Additionally, in case we suspected severe TT obstruction, we planned to perform emergency bronchoscopy for diagnostic and/or therapeutic reasons.

Statistical analysis

Parametric and non-parametric analyses were used in accordance with the results of the Shapiro and Wilk’s W test. Student t-tests, or Wilcoxon-Mann-Whitney U-tests were used to analyse normally and non-normally continuous variables, respectively. The effect of time on daily assessments was assessed with repeated measures one-way ANOVA or Friedman tests. Data are reported either as mean (SD) or as median (interquartile (IQR) ranges) for parametric and non-parametric analyses, respectively. Categorical variables were reported as number of events (%) and analysed using χ² test and Fisher’s exact tests. A two-sided P<0.05 was considered statistically significant. All analyses were performed using SAS 9.2 software.

Results

Population

Nine animals were enrolled into each group; however, data from only eight animals per group were included into the final analysis. In the treatment group, one animal died after 64 h from vomiting and accidental extubation, and it was excluded, as a result of the presence of gastric contents within the TT. In the control group, one animal was euthanized after 30 h for surgical complications, and it was excluded from the analysis because of the short duration of intubation. Another control pig was euthanized after 60 h for sepsis. In the treatment group, one animal died after 64 h from vomiting and it was excluded, as a result of the short duration of intubation. Another control pig was euthanized after 60 h for sepsis. Other complications included hypotension or cardiac dysrhythmias recorded. Additionally, we recorded mean (SD) 5.8 (2.6) and 4.8 (1.6) SEM pictures per TT in the control and treatment group, respectively (

Clinical data

In the treatment and control group, the median (IQR) pulmonary P. aeruginosa burden was 3.34 (1.28) and 4.17 (1.09) log cfu gr⁻¹, respectively (P=0.18). The mean (SD) ratio of arterial oxygen partial pressure to fractional inspired oxygen decreased from 438.3 (43.5) at the beginning of the study to 307.4 (67.1), 349.6 (73.6) and 348.3 (85.1) at 24, 48 and 72 h thereafter, respectively (P<0.001), without differences between study groups. Likewise median (IQR) PEEP was 3 cm (3–5) H₂O and 5 (5–6), 6 (5–7) and 6 (5–7) cm H₂O at 24, 48 and 72 h thereafter, respectively (P<0.001). The mean (SD) number of tracheal suctionings per day was 7.7 (3.7) in the treatment group, and 7.7 (4.5) in the control group (P=0.99). On average, the median (IQR) semi-quantitative amount of aspirated secretions was 2 (1–3) and 2 (2–3) in the treatment and control group, respectively (P<0.001). Overall, in 160 out of 203 aspirations (77.3%) in the treatment group, secretions appeared purulent; whereas, in the control group, 125 out of 173 aspirations (72.2%) purulent secretions were found (P=0.26). Mean (SD) airflow resistance was 8.6 (1.4) in the treatment group, and 8.7 (1.1) cm H₂O litre⁻¹ s⁻¹ in the control (P=0.76).

Tracheal tube bacteriological and microscopy findings

Upon extubation, the median (IQR) gross appearance score was 1 (0–2.5) in the treatment group; whereas, in the control group, it was 3.5 (3–4), P=0.007 (Fig. 2). Of note, in four out of eight control TTs, mucus covered >50% median (IQR) of their lengths. As detailed in Table 1, TT P. aeruginosa colonization did not differ between groups – 5.6 (4.9–6.3) log cfu ml⁻¹ in the treatment group, and 6.2 (5.6–6.9) log cfu ml⁻¹ in the control group (P=0.23). We took 4.0 (1.5) and 3.1 (1.5) CSLM pictures (mean and SD) per TT in the control and treatment group, respectively (P=0.27). As depicted in Figs 3 and 4, the use of the novel CSS decreased biofilm build up. In particular, median (IQR) biofilm area decreased from 4.49 (4.27–4.52) logₐ10 μm² to 3.65 (3.22–4.21) logₐ10 μm² in the control and treatment group, respectively (P=0.031). We recorded mean (SD) 5.8 (2.6) and 4.8 (1.6) SEM pictures per TT in the control and treatment group, respectively (P=0.42). Earlier stages of biofilm development were found in the treatment group in comparison with the control one (Table 2 and Fig. 5).

Feasibility and safety

Overall, the novel CSS was easy to use. In one animal, upon inflation of the catheter’s distal balloon and simultaneous aspiration,
water from the heated humidifier reservoir was suctioned into the ventilatory circuit, because of an inadvertent setting of the suctioning pressure at 400 mm Hg. As a result of the water within the circuit, MV was interrupted and the animal developed severe bradycardia. Brief manual ventilation was necessary to remove the water. Two novel CSSs were replaced after 12 and 26 h of MV, because of accidental tears on the protective plastic sheet. In a control pig (n. 104), the CSS was replaced after 24 h, as a result of a faulty hinge valve. In the same animal, after 70 h of MV, a sudden increase in peak airway pressure and sustained desaturation suggested a significant TT obstruction. Thus, emergency bronchoscopy was performed, which corroborated vast accumulation of secretions within the TT and restored patency.

**Discussion**

This study demonstrated that the novel CSS, in comparison with standard CSS, was efficient in reducing TT biofilm formation and build-up of mucus. The novel CSS was easy to operate and functioned properly throughout the 76-h study period. Finally, when appropriate settings were applied, the novel device did not cause any clinical complications.

In the last decade, several new devices have been developed to remove biofilm and mucus from the TT internal lumen. One of the most innovative is the Mucus Shaver, which comprises an inflatable distal balloon with silicone-rubber rings on its surface. In previous animal studies, the device efficiently cleared TT biofilm and secretions from the TT lumen. In a clinical study, its effectiveness was corroborated and the Mucus Shaver prevented bacterial colonization of the TT. Conversely, in our studies, the novel CSS reduced biofilm formation, but TT colonization was not prevented. Finally, when silver-coated TTs were cleaned with the Mucus Shaver, the anti-bacterial efficacy was retained up to one week of MV. In a study in paediatric settings, investigators have used the inflatable balloon of a urethral catheter to clean the inner lumen of TTs. The use of...
Importantly, the device does not require ventilatory disconnection; it generates a high-pressure effect improved, and biofilm formation, and reduced air flow resistance.31

In our study, the novel CSS was effective in decreasing biofilm and mucus build-up within the TT. Indeed, earlier stages of biofilm development were found in the treatment group. Interestingly, although gross examination of the TT revealed a reduction in mucus build-up, we did not find any decrease in airflow resistance. A potential explanation for these findings is that the accumulation of mucus, during the limited time of our study, may not have been sufficient to increase airflow resistance.31

Importantly, the balloon of the novel CSS does not have rings, which is one of the key features in the design of the Mucus Shaver. These rings strictly adhere against the TT wall, and clear biofilm/mucus.17 This is likely to be unattainable using a standard inflatable balloon, as friction against the TT wall is lessened, and biofilm could be compressed over the TT lumen, rather than removed. Nevertheless, the novel CSS presents major improvements, in comparison with aforementioned apparatus. It generates a high-pressure efflux of saline that is projected against mucus/biofilm, it is reusable for up to 72 h and it allows concomitant displacement and aspiration of biofilm debris. Importantly, the device does not require ventilatory disconnection; potentially decreasing associated risks. Previous studies32–34 have shown that closed suctioning is beneficial in patients with acute respiratory distress syndrome. In this context, closed suctioning prevents reduction of lung volume and desaturation. Indeed, Maggiore and colleagues34 showed that open suctioning caused significant lung volume loss in these patients, half of which was secondary to the ventilator disconnection. It is important to emphasize that in our current study, fewer secretions were aspirated with the novel closed suctioning system. Diameter and location of the catheter side holes could have played a role in these outcomes.35 Additionally, previous investigations35 have shown reduced suctioning efficacy with the use of closed systems compared with open suctioning. Thus, further investigations should characterize these operating limits and lead to potential improvements.

A few features of our settings need to be highlighted. First, we used a model characterized by vast production of purulent secretions. This is an ideal model to test devices aimed at preventing TT biofilm formation. Second, animals were on MV for 76 h to ensure a comprehensive efficacy/safety assessment of the novel CSS. Third, the novel device did not affect pulmonary burden, probably because the infection originated from the lungs, not from the TT as in previous models.3 Theoretically, devices that prevent TT biofilm formation could also reduce relapsing infections caused by biofilm pathogens embedded within the biofilm. This aspect could not have been explored in our settings, but should be prioritized in future investigations. Finally, we used state-of-the-art methods (i.e. quantitative CSLM and SEM) for accurate evaluation of biofilm formation.

As for the safety of the new CSS, as detailed above, a technical problem was encountered during operation. When the CSS balloon is inflated, and aspiration is concurrently activated, the respiratory system is sealed and negative pressure is exerted throughout the ventilatory circuit. Thus, it is important to set the suction pressure as low as possible – ideally less than 200 mm Hg – to avoid accidental aspiration of water from the heated humidifier reservoir. Furthermore, at the beginning of the study, two novel CSSs had to be replaced, because of cuts in the protective sheet. The sheet was promptly redesigned, and this problem never recurred. Finally, in one animal from the control group, we had to perform an emergency bronchoscopy. Full TT obstruction was confirmed and TT patency was restored. In clinical practice, such a situation would be uncommon, but potentially life-threatening.36 Partial obstruction of the TT, however is frequent and leads to increased airflow resistance,37 breathing difficulties and delayed weaning from MV.11 38 This evidence, in addition to future laboratory/clinical

### Table 2 Biofilm stage of recorded scanning electron microscopy pictures per treatment group.

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<thead>
<tr>
<th>Biofilm Stage</th>
<th>Number of Pictures (%)</th>
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<tr>
<td></td>
<td>Control</td>
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<td>0</td>
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<tr>
<td>I</td>
<td>0 (0)</td>
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<tr>
<td>II</td>
<td>11 (68.7)</td>
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<td>III</td>
<td>12 (63.2)</td>
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<tr>
<td>IV</td>
<td>12 (70.6)</td>
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**Fig 4** Quantitative Confocal Scanning Laser Microscopy Analysis. (a) Biofilm area; (b) Biofilm Maximal Thickness; (c) Biofilm Minimal Thickness. In each graph, dot plots depict mean imaging data per each pig. The mean and the median values are indicated by the dashed and dotted lines, respectively and the 25th and 75th percentiles are indicated by the lower and upper light blue horizontal lines. For all analyses, N=16, Wilcoxon-Mann-Whitney U-test.
trials demonstrating reduced incidence of VAP, would support the routine use of these devices.

This study has limitations. First, we used deeply sedated animals, unable to breathe spontaneously. Future investigations should evaluate the feasibility of the novel device during spontaneous breathing. Second, in future studies the novel CSS should be tested against dedicated devices for the removal of TT biofilm. In particular, the device should be compared with the Mucus Shaver, which has consistently demonstrated efficiency in preventing biofilm accumulation, both in laboratory and clinical studies. Third, animals were placed in the lateral-Trendelenburg position, rather than in a supine semirecumbent position as in humans. Our previous investigations have demonstrated improved mucus clearance with the use of the lateral-Trendelenburg position. Thus, we may have overestimated the value of the device, and its effectiveness should be investigated in the supine semirecumbent position. Fourth, we only tested a 12-Fr CSS with a TT of 7.5-mm ID; it is expected that the impact of biofilm/secretions accumulation may depend upon the internal lumen diameter of the TTs. Therefore, testing the usefulness of the device with a range of TT IDs should be evaluated in the future. Finally, our findings need to be corroborated in clinical studies to determine the impact on major clinical outcomes.

In summary, the novel CSS, in comparison with a standard CSS, decreased mucus and biofilm accrual on the TT internal lumen. Additionally, the novel device did not cause any complication when the vacuum was set appropriately. The benefits associated with the routine use of the novel CSS in critically ill patients need to be verified; nevertheless, the clinical usefulness could be substantial, particularly in clearing small ID TTs, and in patients with overproduction of mucus.

Authors’ contributions
Study design/planning: A.X.E., L.B.G., W.D., N.G., T.A.
Data analysis: A.X.E., L.B.G., F.M.
Writing paper: A.X.E., L.B.G.
Revising paper: all authors

Supplementary material
Supplementary material is available at British Journal of Anaesthesia online.

Declaration of interest
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**References**


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