Objective: To determine whether bacterial biofilms are present on pediatric tracheotomy tubes.

Design: Prospective observational series.

Interventions: Eleven tracheotomy tubes removed during routine tracheotomy tube changes were analyzed for biofilm and live bacteria presence using confocal microscopy and vital stains. The external and internal surfaces of the tracheotomy tubes were studied in 3 locations: distal tip, midtracheotomy tube, and proximal opening. These data were correlated with tracheotomy site cultures and the reason for tracheotomy dependence.

Main Outcome Measures: Microscopic images were analyzed for the presence of a biofilm (its morphological features and the presence of live and dead bacteria within the biofilm).

Results: Of 11 tracheotomy tubes, 10 had biofilm present on the internal surface of the distal tip. Externally, at the same location, 4 tubes had biofilms. On the internal surface of the midtracheotomy site, 8 had biofilm present, whereas only 1 had a biofilm on the internal surface of the proximal tracheotomy tube site. In the distal internal tracheotomy tube site, the biofilm was confluent in 5 tubes and patchy with evidence of microcolony formation in the remaining 5 tubes. Live bacteria were present in all biofilms. Control tracheotomy tubes did not have biofilms. All tracheotomy site cultures and disease states (chronic aspiration and bronchopulmonary dysplasia) were associated with tracheotomy tube biofilms.

Conclusion: Bacterial biofilms containing live bacteria were demonstrated in most pediatric tracheotomy tubes, being most frequent and extensive on the internal surface of the distal tracheotomy tip.

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tracheotomy tube hub.

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Figure 1. Tracheotomy tube section sites. Site 1 indicates the distal tip; site 2, the midtracheotomy tube; and site 3, the proximal tracheotomy tube hub.

Table 2. Biofilm Presence on the Internal Tracheotomy Tube Surface

<table>
<thead>
<tr>
<th>Biofilm Variable</th>
<th>Internal Tracheotomy Tube Site</th>
<th>Control Tracheotomy Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Present</td>
<td>10 (91)</td>
<td>8 (73)</td>
</tr>
<tr>
<td>Absent</td>
<td>1 (9)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Total</td>
<td>11 (100)</td>
<td>11 (100)</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) of tubes. Site 1 indicates the distal tip; site 2, the midtracheotomy tube; and site 3, the proximal tracheotomy tube hub.

Eleven tracheotomy tubes (3.5 and 4.0 pediatric Shiley) removed from clinically stable hospitalized patients as a part of routine tracheotomy care were analyzed for the presence of bacterial biofilms. Before removal, all tubes were suctioned. These tubes were treated with humidified air (humidifier temperature setting, 37.5°C) delivered through a tracheotomy mask. No tracheotomy tubes were used in patients who were undergoing mechanical ventilation.

On removal, the tubes were visually inspected for the presence and location of mucus. Following this, the tracheotomy tubes were transected into 1-mm pieces in 3 locations: the distal tip (site 1), the midtracheotomy tube (site 2), and the proximal tracheotomy tube hub (site 3) (Figure 1). Two unused pediatric tracheotomy tubes were subjected to the same procedure, as controls. Sectioning was done under clean conditions with a sterile scalpel and calipers. These tracheotomy tube sections were stored at 0°C and analyzed within 24 hours of removal using fluorescent staining (Live/Dead BacLight bacterial viability kit; Molecular Probes, Eugene, Ore) and confocal microscopy. This kit uses 2 dyes: SYTO9 (green) and propidium iodide (red). The principle behind this technique is the differential permeability of bacterial cell membranes. The green dye is concentrated in live bacteria (ie, an uncompromised cell membrane) and the red in dead bacteria (ie, a compromised cell membrane). Confocal microscopy was chosen for biofilm analysis because it would cause the least biofilm disruption and distortion while allowing the analysis in 3 dimensions.

Imaging was performed using an inverted microscope (Leica DMRBE; Leica Camera AG, Wetzlar, Germany) with a confocal laser scanning system (Leica TCS NT/SP; Leica Camera AG), with excitation lines at 457, 488, 568, and 633 nm at ×5, ×10, and ×25 magnification. Image capture was done using computer software (Leica TCS NT; Leica Camera AG), and images were stored as Tag Image File Format and/or Joint Photographic Experts Group files. Three independent observers (J.P., J.M., and R.P.) analyzed images from each site to determine the biofilm characteristics (patchy or confluent) present at each site. Measures of biofilm greatest thickness in micrometers and biofilm morphological features, such as the presence of towers and the extent of the biofilm coating on the internal and external tube surface, were determined. The biofilm was judged confluent if it completely coated the internal surface of the tracheotomy tube section, and patchy if there were isolated mounds of biofilm. Majority consensus was obtained before reporting findings. In addition, the relative number of live to dead bacteria was determined, in ×25 magnification images, in confluent biofilms using computer software (Adobe Photoshop; Adobe Systems Inc, San Jose, Calif; and Image J; National Institutes of Health, Bethesda, Md). Each image was visually analyzed for live (green) and dead (red) bacteria. Areas containing bacteria staining bright green were selected and isolated from the rest of the image. The area of the selected bacteria was determined with computer software (Image J). The same sequence of events was used for bacteria staining red. A ratio of the area of live bacteria–dead bacteria was determined.

Routine tracheotomy site culture results, when obtained, clinical reasons for tracheotomy, and duration of tracheotomy tube placement were recorded. Descriptive statistics were used for data analysis. The sample size was too small for more rigorous analysis.

### RESULTS

On gross examination, mucus was present on 10 of the 11 study tubes at the internal surface of site 1, with diminishing presence toward the proximal end of the tracheotomy tube. After staining this mucus, it was evident that this was actually a biofilm containing live bacteria. The Table summarizes findings on the internal surface of the tracheotomy tubes at sites 1 through 3. The tube that did not have mucus or a biofilm present had been repeatedly suctioned before removal. No biofilms were present on the unused control tracheotomy tubes.

On the external surface of the study tubes, 4 of 11 had biofilm present at site 1; this decreased to 3 of 11 at site 2 and to 0 of 11 at site 3. The characteristics of patchy
and confluent biofilms are shown in Figure 2 and Figure 3, respectively. At site 1 on the internal tube surface, the biofilm was patchy in 5 of 11 tubes, and the same number of tubes had confluent biofilms. At site 2 on the internal surface, the biofilm was patchy in 6 of 11 tubes and confluent in 2 of 11 tubes. The 1 area of biofilm at site 3 was patchy. There was a wide range of biofilm thickness on the internal surface of site 1 (range, 75.85-1080.80 µm) (Figure 4). The same variation was present on the internal surface at site 2 (range, 20.16-523.07 µm). This reflects the irregular surface of a biofilm and specimen preparation artifact. In those tubes that had confluent biofilms on the internal surface of the tracheotomy tube at site 1, the ratio of live-dead bacteria area was greater than 2 in all cases (range, >2-55).

The tracheotomy tubes were in place for the treatment of chronic aspiration (n=5) and/or structural airway compromise (bilateral vocal cord paralysis or tracheomalacia) (n=6). Tracheotomy site cultures were available in 4 subjects, all of which grew multiple organisms. All tubes from these subjects had biofilm present at site 1, whereas only 2 had biofilm present at site 2. The duration of tracheotomy tube placement ranged from 4 to 30 days (mean, 14 days). There was no correlation between the time that a tracheotomy tube was in place and the extent of the biofilm. The 2 subjects with airway inflammation (aspiration pneumonia and tracheitis) had biofilms at site 1. The duration of tracheotomy tube use and inflammation seemed to result in a thicker and more extensive biofilm at site 1. None of the patients had evidence of clinically significant granulation tissue at the tracheotomy site or at the tracheotomy tube tip.

COMMENT

In the environment, bacteria commonly exist in multispecies biofilms, which have been implicated in dental, otologic, ocular, and nosocomial infection.9-13 Bacterial biofilm formation from multiple organisms has been demonstrated on tracheotomy tube surfaces in vitro and some endotracheal tubes in vivo.14,15 In this preliminary study, bacterial biofilms were demonstrated on pediatric tracheotomy tube surfaces in vivo. The biofilms contained predominately live bacteria and were most prevalent on the internal surface of the tracheotomy tube at site 1 (10 [91%] of the 11 tubes), as has also been seen at the distal tip of endotracheal tubes in patients undergoing ventilation. Conversely, biofilms were present less frequently in the proximal tracheotomy tube. This is possibly due to the high humidity within the trachea compared with a more proximal tracheotomy tube site. Gross inspection of the biofilm on the tracheotomy tube surface also supports this conclusion. Further study of bacterial behavior in the trachea and on tracheotomy tube surfaces is necessary to confirm this impression. The external tracheotomy tube surface did have biofilms present at the distal tip (site 1) (4 [36%] of the 11 tubes), but none were present proximally (site 3). These preliminary findings support the hypothesis that what has traditionally been regarded as mucus within a tracheotomy tube is in reality a bacterial biofilm.

Bacterial biofilm morphological features are related to the microenvironment of the bacteria and the biofilm.16,17 We observed variable biofilm morphological features with confocal microscopy. In all of our specimens, satellite microcolonies, a common means of biofilm enlargement, were present.18 The varied environments within the biofilm allow multiple species and phenotypes of bacteria to survive and thrive, while resisting outside forces that seek their destruction.19 Recently, mathematical modeling of biofilms on solid surfaces has shown that biofilms are similar to other viscoelastic materials that respond to external forces in characteristic ways, resulting

Figure 2. Patchy biofilm at the internal surface of site 1 (original magnification ×10). The bar indicates 84.50 µm; green, live bacteria; and red, dead bacteria.

Figure 3. Confluent biofilm with fluid channels at the internal surface of site 1 (original magnification ×10). Green indicates live bacteria; and red, dead bacteria.
in nonreversible deformation. Subsequent work evaluating biofilms in a high turbulent flow environment (analogous to the end of an open tube) resulted in the growth of elongated filamentous streamers and extrastrong surface adherence that is independent of cell-cell communication within the biofilm. These streamers are thought to break off and cause biofilm spread. In contrast, low turbulent flow results in mound-shaped biofilm microcolonies. These inherent biofilm characteristics may partially explain our finding, and that of others, that biofilms are thickest and most frequent at the distal tip of the tracheotomy tube and then take on a more mound-shaped appearance proximally, where air (the fluid) flow is more laminar. It is unclear why biofilms were more frequent and robust on the internal surface of the tracheotomy tube, compared with the external surface. Possibly, the combination of high humidity, turbulent airflow, and unrestricted space for expansion in the tracheotomy tube lumen explain this finding. Further study is necessary to fully explain this phenomenon.

Interestingly, the time that a tracheotomy tube was in place did not correlate with a more extensive biofilm at any site, possibly because of the mechanical disruption caused by routine tracheotomy tube suctioning. The duration of tracheotomy tube placement and the presence of infection (tracheitis) correlated with increased biofilm thickness at site 1, similar to findings in endotracheal tubes. External tracheotomy site cultures were correlated with the presence of biofilms, but not with biofilm involvement of more than 1 tracheotomy tube site. However, in analyzing all these variables, the sample size limits the conclusions that can be drawn from these preliminary data.

There are obvious shortcomings to this study. Our sample size was small, limiting the conclusions that can be made from our data. A more careful study of bacterial flora within and around the tracheotomy tube would be helpful in delineating the bacterial ecological features in this location. Cultures of various tracheotomy tube sites, and genetic identification of bacteria within and adjacent to the biofilms, would allow determination of the exact type of bacteria present in the biofilm, potentially allowing organism-specific therapy. Bacterial adherence to solid surfaces in aqueous environments is rapid and, consequently, most of our specimen had evidence of mature biofilms. A prospective evaluation of tracheotomy tubes removed at set times would allow for a description of the timing of bacterial adherence and biofilm formation on tracheotomy tubes.

What are the clinical implications of these findings? Tracheotomized pediatric patients have long been plagued by localized mixed bacterial infections because of the disruption of the innate cutaneous and mucosal immunity by the tracheotomy. It seems that biofilm formation allows the persistence of bacteria on tracheotomy tubes and may contribute to chronic inflammation in this area. The insertion of a solid surface through this opening results in a place for bacterial adherence. Bacterial biofilms commonly have more than 1 bacterial species present. Biofilms shield bacteria from antibiotics, humoral and oxidative mechanisms of bacterial destruction, enabling bacterial persistence and multiplication. In addition, increasing humidification in the tracheotomy tube is a part of routine tracheotomy care, but the impact of this treatment on biofilm formation is unknown. It is possible that increasing tracheotomy tube humidification could enhance biofilm formation because of the creation of a more aqueous environment. However, the reduction of humidification is known to be associated with tracheotomy tube occlusion. In either scenario, a novel therapy directed at reducing the thickness and prevalence of the biofilm within the tracheotomy tube may aid in preventing tracheotomy plugging and reducing tracheotomy site inflammation. If this is true, then therapies to penetrate, disrupt, or otherwise disable the biofilm more effectively than current methods could improve tracheotomy care. For example, evidence that pieces of dislodged biofilm cause peripheral pneumonic infection in patients undergoing ventilation could also be important in tracheotomy suctioning techniques and catheters. These techniques focus on the reduction of tracheal trauma, without concern for biofilm disruption. Maybe suctioning techniques and devices would be more effective if they were specifically directed at complete biofilm disruption and removal. As mechanisms of bacterial genetic and biochemical control of biofilm formation, spread, and maintenance are understood, insight into new therapeutic methods to treat problems created by biofilm formation will occur. Further study will be necessary to determine whether prevention of bacterial adherence or reduction of biofilm formation will lead to decreased local tracheotomy tube morbidity, ultimately improving tracheotomy care.

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REFERENCES