Effectiveness of ethylene oxide for sterilization of dental handpieces

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ABSTRACT
Ethylene oxide gas has been utilized as an alternative method for sterilization of dental handpieces, as it is less corrosive than steam. However, its effectiveness for sterilization of the internal components of dental handpieces has not been established. The objective of this study was to compare the effectiveness of ethylene oxide and steam for sterilization of dental handpieces. Unused handpieces and handpieces which had been exposed to clinical dental procedures ('clinical') were contaminated with Streptococcus mutans exposed to steam or ethylene oxide, and flushed with sterile saline. Washings were plated on mitis-salivarius agar, and colonies identified and counted. No viable colonies could be established from washings from 'clinical' or 'unused' handpieces exposed to steam. However, viable colonies could be established from 'clinical' handpieces exposed to ethylene oxide. This data suggests that a substance entrapped within 'clinical' handpieces (possibly the biofilm) may protect bacteria from ethylene oxide gas, preventing adequate sterilization.

KEY WORDS: Ethylene oxide, Biofilm, Dental handpiece, S. mutans

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INTRODUCTION
High-speed dental handpieces are known to collect particulate matter and bacteria from the mouth and from the water supply to the dental unit. As these microorganisms could be transferred to patients, sterilization of both internal and external handpiece surfaces is required to prevent iatrogenic cross-infection. While sterilization of external surfaces is not difficult, achieving adequate sterilization of internal surfaces is complicated by several factors associated with handpiece design; that is, various lumens and crevices which collect microorganisms and debris are difficult to properly clean and sterilize.

The most commonly used procedure for sterilization of dental handpieces is exposure to steam under pressure in an autoclave. However, metallic and non-metallic components of handpiece turbines are reported to be adversely affected by steam which results in progressive deterioration of their speed. Thus, institutions which use large numbers of handpieces, such as dental schools, hospitals and large dental clinics, consider alternatives to steam sterilization: in particular, use of ethylene oxide gas. Ethylene oxide gas is capable of sterilization of instruments and other contaminated objects because of its excellent penetration properties and its ability to kill spore-forming bacteria, viruses and fungi at relatively low temperatures. These latter effects result from its ability to alkylate essential cellular proteins which adversely affect cellular metabolism. However, there is little information about the ability of ethylene oxide gas to sterilize the lumens of dental handpieces, although a study of the potential use of this gas to sterilize laparoscopic equipment (which also contains internal crevices and lumens) suggests that these instruments must be disassembled and cleaned prior to exposure to ethylene oxide gas to achieve sterility. Disassembly of dental handpieces could be either difficult or impossible to perform in the dental office, depending on their design. The objective of this investigation is to study the ability of ethylene oxide gas to sterilize internal surfaces of handpieces inoculated with Streptococcus mutans, a
Table 1. Mean number of bacterial colonies (+ s.e.m.) derived from washings from 'clinical' and 'unused' handpieces following sterilization by ethylene oxide and steam

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ethylene oxide</th>
<th>Steam</th>
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<tbody>
<tr>
<td>'Clinical'</td>
<td>226.8 ± 11.0**</td>
<td>0</td>
</tr>
<tr>
<td>'Unused'</td>
<td>0.4 ± 0.1*</td>
<td>0</td>
</tr>
</tbody>
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Control = 286.1 ± 12.4 colonies 60 mm dish⁻¹. Significantly less than control: *P < 0.001, **P < 0.05. Significantly less than 'clinical': ¹P < 0.001.

common contaminant of the oral cavity. This information will suggest whether use of this gas is a viable alternative to steam for sterilization of dental handpieces by institutions which utilize large numbers of handpieces.

MATERIALS AND METHODS

Microorganisms

Streptococcus mutans was obtained from the American Type Culture Collection, Rockville, Maryland. Pure cultures were grown and maintained in trypticase soy broth at 37°C.

Agar

These experiments utilized a selective medium for Streptococcus mutans originally described by Gold and coworkers¹⁹. Mitis-salivarius agar containing 0.2 units ml⁻¹ bacitracin, 20% sucrose and 0.001% tellurite was prepared and placed in 60 mm petri dishes. This selective media has been extensively used, as it promotes the growth of S. mutans and inhibits growth of other forms of streptococcus.

Dental handpieces

Eighty-four air turbine high-speed handpieces were used for this study. Forty-two handpieces were selected from those in clinical use and were classified as 'clinical' herein. Forty-two handpieces had never been used in the dental clinics and were classified as 'unused'. Prior to experiments, handpieces were flushed with air and water as suggested by Scheid et al. and air dried. Handpieces were inoculated through the air tube with 0.5 ml phosphate-buffered saline (PBS) containing 10⁶ S. mutans, dried at 37°C for 45 min, and placed in autoclave bags. One-half (21 'clinical' and 21 'unused') of the handpieces were then placed in a 3M Steri-Vac 400XL ethylene oxide sterilization unit and exposed to ethylene oxide gas as recommended by the manufacturer and by previous investigations¹³. One-half (21 'clinical' and 21 'unused') were exposed to steam at 250°C at 20 psi (138 kPa) for 20 min. All handpieces were removed from the autoclave bags and flushed with 0.5 ml sterile PBS, which was collected in a petri dish containing the mitis-salivarius agar. For 'control' cultures, agar was exposed to either 0.5 ml sterile PBS or 10⁶ microorganisms suspended in 0.5 ml PBS. Cultures were grown in an atmosphere of 95% nitrogen and 5% carbon dioxide for 5 days and photographed. Bacterial colonies were counted from photomicrographs at 3X magnification. The mean number of colonies was determined by each group and compared by factorial analysis of variance and a post-hoc Tukey's test.

RESULTS

Numerous, large bacterial colonies were evident on 'control' agar plates inoculated with 10⁶ microorganisms. There were significantly more colonies on these plates than in other groups (Table I). No bacterial colonies were evident following inoculation, flushing and plating of bacteria from the 21 'unused' and 21 'clinical' handpieces which were steam sterilized. Bacterial colonies could be established from washings from all 21 'clinical' handpieces exposed to ethylene oxide (Table I). A small number of viable colonies could also be established from washings from several 'unused' handpieces exposed to ethylene oxide (Table I).

DISCUSSION

An ideal sterilizing agent for dental handpieces would be a non-corrosive gas which was readily diffusible and could destroy all forms of microorganisms at ordinary temperatures. Ethylene oxide gas fulfills some of these requirements; however, our data suggests that it might not be able to destroy microorganisms within dental handpieces following standard methods for preparation of handpieces for sterilization.

Our study demonstrates that ethylene oxide gas cannot destroy all viable S. mutans in handpieces which have been used for clinical procedures; however, it can destroy most viable S. mutans within previously unused handpieces. Thus, since S. mutans is not usually difficult to destroy, there is probably a material which accumulates within dental handpieces exposed to clinical dental procedures which is not readily removed by flushing with water and protects inoculated bacteria from sterilizing effects of the ethylene oxide gas. The presence of crystalline or dry proteinaceous material¹⁴ and serum¹¹ has been reported to hinder the efficiency of ethylene oxide gas for sterilization. This biofilm has been reported to accumulate from aspiration of bacteria, saliva and ground hydroxyapatite and dental filling materials into the internal crevices of the handpiece and may not be readily removed by flushing with water. In particular, when a handpiece lubricant has been used¹³, n. In addition, flushing of the handpiece with water from the dental unit may introduce additional contaminants from a contaminated water line³⁻⁷.

Thus, sterilization of dental handpieces by ethylene oxide gas is probably not possible without removal of the biofilm from the internal lumens and crevices. This is also evident in sterilization of other medical instruments with lumens (such as the laparoscope), which must be disassembled and cleaned before sterilization by ethylene oxide gas is possible. Since high-speed dental handpieces may be difficult (or impossible) to disassemble, alternative methods for cleaning of internal surfaces must be developed for ethylene oxide to be a viable method for dental handpiece sterilization.

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References