Effects of sterilization with hydrogen peroxide gas plasma, ethylene oxide, and steam on bioadhesive properties of nylon and polyethylene lines used for stabilization of canine stifle joints

Matthieu Gatineau, DMV; Alexander O El-Warrak, DMV, PhD; Christian Bolliger, Dr med vet; Michael Mourez, PhD; Frederic Berthiaume, MSc

Bacterial contamination of biomaterial surfaces during surgery is the primary cause of implant-related infections. Results of several studies have clearly indicated that sterilization can affect surface properties of polymers and, consequently, their adhesive properties. Rupture of the cranial cruciate ligament is one of the most commonly diagnosed orthopedic conditions in dogs and leads to patient morbidity, reduced activity, and progressive degenerative joint disease. Surgical stabilization of the stifle joint is recommended, especially in large-breed dogs. Extra-articular stabilization with a lateral suture is a well-accepted and commonly performed technique, and many materials have been used for the lateral suture technique. However, to the authors knowledge, no studies have been conducted to evaluate the effect of various sterilization methods on bioadhesive properties of those materials.

Compared with steam sterilization, ethylene oxide sterilization was found to have the least detrimental effects on mechanical characteristics of lateral suture materials. However, studies in humans suggest that ethylene oxide could be carcinogenic and associated with breast and other cancers in exposed individuals. The use of ethylene oxide sterilization in North America has diminished because of strict regulations. Sterilization with HPGP is an attractive alternative to low-temperature sterilization with ethylene oxide. The HPGP sterilization is accomplished through synergism

Objective—To compare effects of sterilization with hydrogen peroxide gas plasma (HPGP), ethylene oxide, and steam on bioadhesive properties of nylon and polyethylene lines used for stabilization of canine stifle joints.

Sample—Samples of a 36.3-kg test nylon leader line, 57.8-kg test nylon fishing line, and 2-mm ultrahigh–molecular weight polyethylene (UHMWPE) were used.

Procedures—In this in vitro study, samples of nylon leader line, fishing line, and UHMWPE sterilized by use of HPGP, ethylene oxide, and steam or unsterilized samples were used. Bacterial adherence on unsterilized and sterilized samples was tested with Staphylococcus epidermidis and Escherichia coli. Five samples were examined for each line type and sterilization condition, and final colony counts were obtained.

Results—Bacterial adherence was significantly affected by method of sterilization for all 3 line types. For most of the samples, bacterial adherence was similar or lower when HPGP sterilization was used, compared with results for sterilization via ethylene oxide and steam, respectively. Bacterial adherence was significantly higher for UHMWPE, compared with adherence for the nylon line, regardless of the sterilization method used. Bacterial adherence was higher for nylon fishing line than for nylon leader line for S epidermidis after ethylene oxide sterilization and for E coli after HPGP and ethylene oxide sterilization.

Conclusions and Clinical Relevance—Effects of HPGP sterilization on bioadhesive properties of nylon and polyethylene lines compared favorably with those for ethylene oxide and steam sterilization. Also, nylon line may be a more suitable material than UHMWPE for suture prostheses on the basis of bacterial adherence properties. (Am J Vet Res 2012;73:1665–1669)
between hydrogen peroxide and low-temperature gas plasma. Toxic residues or emissions have not been reported; thus, aeration, ventilation, and other special procedures are not required.29 System by-products are oxygen and water vapor, and operators never come in contact with hazardous materials because the system involves easy-to-insert cassettes. Investigators of a recent study25 reported that HPGP sterilization was an attractive alternative to ethylene oxide sterilization with regard to mechanical properties of nylon lines used for latural suture techniques.

The objective of the study reported here was to compare the effect of HPGP, ethylene oxide, and steam sterilization on the bacterial adherence properties of materials currently used for extra-articular stabilization of cranial cruciate ligament–deficient canine stifle joints. We hypothesized that HPGP sterilization would perform as well as ethylene oxide or steam sterilization.

Materials and Methods

Sample—Nylon leader line, nylon fishing line, and UHMWPE were selected for evaluation because of their wide application in small animal orthopedic procedures. Samples of 36.3-kg test monofilament nylon leader line, a 57.8-kg test monofilament nylon fishing line, and 2-mm UHMWPE were examined.

Sterilization procedures—Samples (length, 10 cm) of each line type were sterilized. The HPGP sterilization was performed as recommended by the manufacturer for 28 minutes. Ethylene oxide sterilization was performed as recommended by the manufacturer at 55°C and 56 kPa. Steam sterilization was performed as recommended by the manufacturer at 121°C and 110 kPa with an autoclave. After ethylene oxide sterilization, aeration was allowed for 12 to 24 hours as recommended; thus, aeration, ventilation, and other special procedures are not required. System by-products are oxygen and water vapor, and operators never come in contact with hazardous materials because the system involves easy-to-insert cassettes.

Evaluation of bacterial adherence—Gram-positive and -negative bacteria were used to evaluate bacterial adherence on samples. A well-characterized biofilm-producing Staphylococcus epidermidis strain (ATCC 35984) was used as the gram-positive bacteria. Escherichia coli K12 strain C600 containing the pAGH or pTrc99a vectors described in another study27 was used as the gram-negative bacteria. The pAGH vector codes for the AIDA-I autotransporter, which allows the production of a biofilm; pTrc99a is a control empty vector.

Staphylococcus epidermidis strains were inoculated into brain-heart infusion broth containing 0.25% glucose with a turbidity of 0.5 McFarland units. Escherichia coli strains were grown without agitation for 24 hours at 30°C in M9 medium containing 0.005% proline, leucine, and threonine and 100 µg of ampicillin/mL. One milliliter of the normalized cultures was placed in the wells of a 24-well plate that contained a section of the various nylon and polyethylene lines (each section was 1 cm in length; 5 replicates for each line type and sterilization condition). Plates then were incubated overnight at 37°C. At the end of the incubation period, each of the sections was removed from the medium and washed 3 times with 1 mL of PBS solution to remove nonadherent microorganisms.

Adhered S epidermidis or E coli biofilms were separated from the surface of the nylon and polyethylene lines by the addition of 1 mL of PBS solution (for S epidermidis) or 1 mL of PBS solution containing 0.5M NaCl and 1% Triton X-100 (for E coli), which was followed by vortexing for 10 minutes at room temperature (approx 27°C). The bacteria were diluted in PBS solution, and 100 µL of each diluted bacterial suspension was plated on Luria-Bertani agar (for S epidermidis) or Luria-Bertani agar containing 100 µg of ampicillin/mL (for E coli). After incubation for 24 hours, the number of CFUs were counted and adjusted on the basis of the dilution factor.

Statistical analysis—A 2-way ANOVA, with line type and sterilization condition as main factors and the interaction between the 2 main factors, was used to examine the effect of sterilization method on bacterial adherence for each material. A post hoc Tukey test was used to determine differences between pairs of means. A statistical program3 was used for all analyses. Values of P < 0.05 were considered significant.

Results

The mean ± SD log₈₀ number of CFUs of S epidermidis and E coli was determined for each line type and sterilization condition.

Number of CFUs of S epidermidis—The mean number of CFUs was significantly higher (P < 0.001) after sterilization of UHMWPE with HPGP, ethylene oxide, and steam, compared with the number of CFUs for unsterilized UHMWPE (Table 1). There was no significant difference in the number of CFUs among sterilization methods.

The mean number of CFUs did not differ significantly between unsterilized nylon leader line and nylon leader line sterilized with HPGP (Table 1). However, the mean number of CFUs was significantly lower for nylon leader line sterilized with ethylene oxide (P < 0.001) or steam (P = 0.009), compared with results for unsterilized nylon leader line. Steam-sterilized nylon leader line had a significantly (P = 0.008) higher mean number of CFUs, compared with results for HPGP-sterilized nylon leader line, which had a significantly (P < 0.001) higher mean number of CFUs than the number for ethylene oxide–sterilized nylon leader line.

The mean number of CFUs did not differ significantly between unsterilized nylon fishing line and nylon fishing line sterilized with HPGP or ethylene oxide (Table 1). The mean number of CFUs was significantly (P < 0.001) higher after steam sterilization of nylon fishing line, compared with results for unsterilized nylon fishing line. Steam-sterilized nylon fishing line had a significantly (P < 0.001) higher mean number of CFUs, compared with the mean number of CFUs for HPGP- and ethylene oxide–sterilized nylon fishing line.
There was no significant difference in the mean number of CFUs between HPGP-sterilized and ethylene oxide–sterilized nylon fishing line.

The mean number of CFUs for UHMWPE was significantly \((P < 0.001)\) higher than that for both nylon lines for each of the sterilization conditions (including unsterilized). Nylon fishing line had a significantly \((P = 0.021)\) higher mean number of CFUs, compared with results for nylon fishing line, after ethylene oxide sterilization. There were no significant differences between nylon fishing line and nylon leader line when they were unsterilized or after HPGP and steam sterilization.

Number of CFUs of \(E\) coli—The mean number of CFUs did not differ significantly between unsterilized UHMWPE and HUMWPE sterilized with HPGP or ethylene oxide (Table 2). However, UHMWPE sterilized with steam had a significantly higher mean number of CFUs, compared with the number for UHMWPE sterilized with HPGP \((P < 0.001)\) or unsterilized UHMWPE \((P = 0.004)\). There was no significant difference in the mean number of CFUs between UHMWPE sterilized with ethylene oxide or steam.

The mean number of CFUs did not differ significantly between unsterilized nylon leader line and nylon leader line sterilized with steam (Table 2). The mean number of CFUs was significantly lower for nylon leader line sterilized with HPGP \((P = 0.002)\) or ethylene oxide \((P < 0.001)\), compared with results for unsterilized nylon leader line. Steam-sterilized nylon leader line had a significantly higher mean number of CFUs, compared with the mean number of CFUs for HPGP- and ethylene oxide–sterilized nylon leader line. There was no significant difference in the mean number of CFUs for nylon fishing line, after ethylene oxide sterilization, compared with results for nylon fishing line and nylon leader line after steam sterilization.

### Table 1—Mean \(\pm SD\) log10 number of CFUs* of \(Staphylococcus\) \(epidermidis\) on nylon and polyethylene lines that were not sterilized or that were sterilized by use of HPGP, ethylene oxide, and steam.

<table>
<thead>
<tr>
<th>Line type</th>
<th>Unsterilized</th>
<th>HPGP</th>
<th>Ethylene oxide</th>
<th>Steam</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHMWPE</td>
<td>5.77 ± 0.99c</td>
<td>6.63 ± 0.09a</td>
<td>6.63 ± 0.07c</td>
<td>6.78 ± 0.09a</td>
</tr>
<tr>
<td>Nylon leader line</td>
<td>5.27 ± 0.21d</td>
<td>5.22 ± 0.16c</td>
<td>4.75 ± 0.18c</td>
<td>5.04 ± 0.09a</td>
</tr>
<tr>
<td>Nylon fishing line</td>
<td>4.98 ± 0.08c</td>
<td>5.07 ± 0.10a</td>
<td>5.10 ± 0.19c</td>
<td>5.73 ± 0.15c</td>
</tr>
</tbody>
</table>

*Values reported represent results for 5 replicates for each line type and sterilization condition.

Values with different superscript letters differ significantly \((P < 0.05)\).

### Table 2—Mean \(\pm SD\) log10 number of CFUs* of \(Escherichia\) \(coli\) on nylon and polyethylene lines that were not sterilized or that were sterilized by use of HPGP, ethylene oxide, and steam.

<table>
<thead>
<tr>
<th>Line type</th>
<th>Unsterilized</th>
<th>HPGP</th>
<th>Ethylene oxide</th>
<th>Steam</th>
</tr>
</thead>
<tbody>
<tr>
<td>UHMWPE</td>
<td>5.27 ± 0.05c</td>
<td>5.13 ± 0.09e</td>
<td>5.47 ± 0.04a</td>
<td>5.56 ± 0.04e</td>
</tr>
<tr>
<td>Nylon leader line</td>
<td>4.50 ± 0.14d</td>
<td>4.20 ± 0.09c</td>
<td>4.08 ± 0.11c</td>
<td>4.74 ± 0.09c</td>
</tr>
<tr>
<td>Nylon fishing line</td>
<td>4.18 ± 0.14c</td>
<td>4.63 ± 0.17a</td>
<td>4.75 ± 0.12c</td>
<td>4.75 ± 0.08c</td>
</tr>
</tbody>
</table>

*Values with different superscript letters differ significantly \((P < 0.05)\).

See Table 1 for remainder of key.

### Discussion

Sterilization methods used for polymers in the medical field should be considered when developing new products because they may adversely affect or enhance the properties of surgical implants.\(^1^\)\(^,\)\(^2^\)\(^,\)\(^3^\) The HPGP sterilization method was compared with ethylene oxide and steam sterilization of nylon and polyethylene lines used to stabilize canine stifle joints. Analysis of results of the present study indicated that HPGP sterilization compared favorably with ethylene oxide or steam sterilization of nylon and polyethylene lines with regard to the bacterial adherence properties of \(S\) \(epidermidis\) and \(E\) \(coli\). Furthermore, UHMWPE had higher bacterial adherence, compared with bacterial adherence for nylon leader line and nylon fishing line.

In 1 study,\(^10\) ethylene oxide sterilization was considered generally more efficient than HPGP sterilization in preventing bacterial adherence to UHMWPE. In the present study, effects of HPGP on the bacterial adhesive properties of UHMWPE, nylon leader line, and nylon fishing line compared favorably with the effects of ethylene oxide, which would indicate that HPGP is an effective alternative method for these materials. Moreover, bacterial adherence was higher after steam sterilization for almost all samples tested with \(S\) \(epidermidis\) and \(E\) \(coli\). On the basis of these findings, steam sterilization could represent a less suitable sterilization method than HPGP or ethylene oxide sterilization for polyethylene and nylon lines.

It has been clearly determined that the presence of suture material in host tissue increases the susceptibility to infection.\(^11\) Bacterial adherence to biomaterial surfaces is an important step in the pathogenesis of prosthetic-related infection.\(^12^\)\(^,\)\(^13\) Numerous studies have been conducted to examine bacterial adhesion and colonization on a variety of biomaterials. In general, such as type of organism, concentration and growth phase of the organism, and surface properties of the material will affect the amount of colonization and the formation of biofilm.\(^15^\)\(^,\)\(^16^\)\(^,\)\(^17\)\(^,\)\(^30^\)\(^,\)\(^34\)

AJVR, Vol 73, No. 10, October 2012  1667

Unauthenticated | Downloaded 09/20/22 06:03 PM UTC
The method of sterilization may influence bacterial adherence, as indicated in the study reported here, and ethylene oxide and HPGP sterilization may alter the surface and biomechanical properties of polymers, at least temporarily. In the present study, UHMWPE had increased adhesion of bacteria, compared with results for nylon leader line and nylon fishing line. Another variable that considerably influences bacterial adhesion is the roughness of the substrates’ surface. In 1 study, the roughness and hydrophobicity of the surface of the UHMWPE were increased, compared with roughness and hydrophobicity of the surface of nylon leader line and nylon fishing line, regardless of the sterilization method used (HPGP, ethylene oxide, or steam). A rougher surface provides a larger area for bacterial adherence and multiplication and biofilm formation.

Bacteria adhering to irregular surfaces are protected against mechanical forces, even during the initial phase of reversible adherence. Bacterial adhesion as well as colonization is increased on biomaterials with rougher surfaces. In addition, UHMWPE is considered to be hydrophobic, which facilitates bacterial contact and leads to increased early adherence. These findings suggested that when ethylene oxide is used for sterilization, nylon leader line may be a more suitable material than nylon fishing line on the basis of bacterial adherence properties. However, in vivo factors (ie, the presence of adhesive molecules, dissolved proteins, and type of host tissue) also influence initial bacterial adhesion. In the present study, we examined in vitro some specific bacterial strains that may be involved in the pathogenesis of implant-related infection in a clinical setting.

Gram-positive and gram-negative bacteria were used in the present study. Even though it was not specifically examined, S. epidermidis typically adheres to nylon and polyethylene lines more than does E. coli. Differences in the bacterial wall may result in variation in affinity to a surface and should be considered when the bioadhesive properties of a polymer are evaluated. The cell envelope of a gram-positive bacterium such as S. epidermidis consists of a single layer of peptidoglycans. The cell envelope of a gram-negative bacterium such as E. coli is a multilayered, complex structure comprising an outer lipidic membrane with lipopolysaccharides and proteins. In another study, bacteria that were more hydrophobic had increased adherence to the test material. Factors that may influence early bacterial adhesion to polymer surfaces in vitro include the type of culture medium, culture conditions, incubation time, and growth phase of the bacteria. In the present study, both strains were identically prepared to eliminate the effect of difference in growth conditions. Although many methods for counting bacteria have been described, indirect counting methods, such as the number of CFUs on a plate, are widely used, and a CFU plate count is one of the most popular methods. This technique is sensitive and has the advantage of providing counts for only live bacteria. However, investigators should consider that there are several disadvantages of this technique (only live bacteria develop colonies that are counted, and clumps or chains of bacteria that develop into a single colony cause a gross underestimation of the true bacterial population). In addition, experimental manipulations (especially during the dilution and plating steps) could potentially increase the risk of errors.

Analysis of the results of the present study indicated that the effects of HPGP sterilization on the bacterial adhesive properties of nylon and polyethylene lines compared favorably with those for ethylene oxide or steam sterilization, which makes HPGP an attractive alternative method for sterilization. In addition, bacterial adhesion of S. epidermidis and E. coli was more extensive on UHMWPE than on nylon leader line or nylon fishing line. Therefore, UHMWPE may be a less suitable material than nylon line for use as prostheses on the basis of bacterial adherence properties. Studies are needed to confirm the clinical relevance of our findings.