Ultrasonic Cleaning of Special Instruments

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OBJECTIVE

The objective of this study was to measure the ultrasonic cleaning of four brands of dental curettes.

METHODS

These procedures were patterned after previous work that studied instrument cleaning in an ultrasonic cleaner (1) and in a washer-disinfector (2). However, in this study removal of the blood was measured assaying for protein on cleaned and uncleaned instruments in addition to using the Hemastix assay for blood. Procedures for recovering and measuring protein have been modified from a previous report (3). Protein remaining on the instruments after cleaning will be quantitatively measured by using the Lowry method (J Biol Chem 1951; 193:265) of protein analysis. This method detects a wide range of proteins and glycoproteins.


Summary

Four brands of dental curettes were contaminated with sheep blood containing hydroxyapatite by completely submerging in the mixture. After standing at room temperature for 1 hour the instruments were placed in an ultrasonic cleaning basket and the basket placed in the ultrasonic cleaner containing ultrasonic cleaning solution. The unit was operated for 90 sec, 120 sec or 300 sec. Each run contained 5 of each of the four brands of instruments for a total of 20 instruments. Three runs were made at each time period. Fresh cleaning solution was used for each run. The instruments were rinsed in a standardized fashion and analyzed for remaining contaminants by visual observation, the Hemastix assay for blood and quantitatively for protein. Control instruments were contaminated but not cleaned and were analyzed for protein.

Rinsing Test Instruments

Rinsing under a faucet is very difficult to standardize and can effect the results of the blood/protein assay. Thus the processed test instruments were rinsed in a standardized procedure by carefully removing the cleaning basket from the cleaning chamber and letting it drain on paper towels for 10 sec. The basket was then dipped two times into each of four separate two-liter amounts of fresh tap water at room temperature. The rinsed test instruments were then analyzed for blood and protein as described below.

Processing Control Instruments

Control instruments consisted of two sets of 5 instruments of each brand. The control instruments were contaminated with the blood-HA and dried just like the test instruments but were not processed through the ultrasonic cleaner. These control instruments were analyzed by the same procedures used to analyze test instruments and used to calculate % protein removal.

Analysis for visible blood

Each instrument was visually observed with the naked eye for the presence of blood. These data are presented as the number of instruments with visible blood over the total number of instruments analyzed.

Total instrument contamination

The blood-HA mixture was placed in a sterile pan and each test and control instrument was completely submerged in the blood-HA for 10 seconds. Each instrument was removed from the blood and held vertical for 10 seconds to drain. All contaminated instruments were placed in a covered drying rack with minimal contact with the contaminated surfaces at room temperature for 1 hour before being placed into the instrument basket for cleaning (test) or before being directly analyzed for blood protein without cleaning (controls).

Materials

An L & R Ultrasonic cleaner (Quantrax S310) with a stainless basket was used along with Health Sonics All-purpose Cleaning Solution diluted with 40 parts of tap water as indicated on the product label. Defibrinated sheep blood was obtained form Colorado Serum Co. Denver, CO. It was mixed with hydroxyapatite at 50 mg/mL obtained from Sigma Chemical, St. Louis, MO. Prophy brushes (#201) were used to remove the remaining blood from instruments. All of these items were supplied by Indiana University. The Sponsor provided 10 each of the 4 brands of curettes from American Eagle (Code A), Dentsply (Code B), Hu-Friedy (Code C) and Lasco (Code D) (Figure 1). In the tables these are identified as A, B, C, and D, respectively. All instruments were thoroughly cleaned before they were used and reused in this study.

Instrument Contamination with Blood and Hydroxyapatite

Both test and control curettes were contaminated with a mixture of blood and hydroxyapatite (HA). The insoluble HA simulated tooth structure. One batch of defibrinated sheep blood was used. HA was added to the blood at 50 mg per mL with stirring before each use.

Figure 1: Test instruments used
(From top to bottom: A, American Eagle; B, Dentsply; C, Hu-Friedy; D, Lasco)
Hemastix assay for blood in the SDS recovery fluid

The presence of blood in the SDS recovery fluid was qualitatively measured by using Hemastix®. This is a chemical-coated plastic strip that changes color when exposed (dipped into the SDS) to detect as little as about one ppm of blood. The intensity of the color on the strip is visually compared to a color chart to indicate the relative amount of blood present as None, Trace, Small, Moderate or Large. To add a quantitative aspect to this assay, numerical values were assigned to each visualization as follows: Trace = 1; Small = 2; Moderate = 3; Large = 4. The number of instruments positive for blood over the total number of instruments analyzed is presented along with the average numerical values for each set of 15 instruments (5 instruments in 3 runs).

Assay for blood protein in the SDS recovery fluid

The SDS recovery fluid was quantitatively assayed for the presence of protein using the Lowry method described below. These data are presented in three ways: 1) the number of instruments containing any amount of protein over the total number of instruments analyzed; 2) as % of protein per mL; 3) the average amount recovered from each set of 15 test instruments (5 instruments in 3 runs) is compared to the average amount of protein recovered from the uncleaned control instruments, and the % protein removal from the test instruments was calculated. This Lowry method is able to detect as little as about 30 g (an invisible amount) of protein per instrument. Bovine serum albumin (Fraction V, #A-4503, Sigma Chemical Co, St. Louis, MO) will be used as the protein to standardize the assay for each batch of samples analyzed.

Lowry method to detect protein (J Biol Chem 1951; 193:265)
1. 0.6ml of sample
2. add 3.0 mL of reagent E and mix
3. let stand 10 min at room temperature
4. add 0.3 mL of reagent F with rapid and immediate mixing
5. let stand 30 min at room temperature
6. read absorbancy at 690 nm using a spectrophotometer

Known amounts of bovine serum albumin fraction V will be used as the standard in the assay in order to calculate the number of g protein present in the SDS recovery fluid samples. SDS does not interfere with this assay.

Reagents:
- Reagent A: 2.0% Na2CO3 in 0.1N NaOH
- Reagent B: 0.5% CuSO4 - 5H2O in deionized water
- Reagent C: 1.0% sodium - potassium tartarate in deionized water
- Reagent D: equal volumes of Reagents B and C
- Reagent E: 50.0 mL and Reagent A plus 1.0 mL of Reagent D (prepare fresh daily)
- Reagent F: Phenol reagent (Folin-Ciocalteau reagent) at 1.0 N

**RESULTS**

Table 1 presents the results of protein recovery from the four types of instruments that were contaminated but not cleaned. The average amount of blood protein recovered from the four types of uncleaned control instruments did not significantly differ. These data were used to calculate the percent protein removal from the test instruments.

Table 2 presents the results from processing test instruments thought the ultrasonic cleaner for various cleaning times.

**Visual blood**

The measure of instrument cleanliness in the dental facility is the visual absence of debris. In this study the visual absence of blood on all instruments in all groups occurred only after 300 seconds of ultrasonic cleaning.

**Hemastix assay for blood**

The Hemastix assay is very sensitive and can detect blood that is not visible with the naked eye. This assay detected varying amounts of blood on all instruments at all time periods except instrument D at 300 sec where there were 5 of the 15 instruments that were free of detectable blood. The relative amount of blood present on the positive instruments is presented. Instrument D had the lowest average level of detectable blood, and this difference was statistically significant at all three time periods.

**Protein**

Instrument D showed the fewest number of instruments containing detectable protein at 90 sec, 120 sec and 300 sec. The average amount of protein detected from each of the four groups of instruments was the lowest for instrument D at 60 sec and at 300 sec. At 120 sec the lowest average amount was recovered from instrument B. All instruments showed better than an average of over 99% removal of protein at all cleaning times when compared to the uncleaned control instruments.

Table 1. Controls

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Protein Present (μg/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3151.00 ± 279.94**</td>
</tr>
<tr>
<td>B</td>
<td>3346.00 ± 458.80**</td>
</tr>
<tr>
<td>C</td>
<td>3276.00 ± 408.77**</td>
</tr>
<tr>
<td>D</td>
<td>3070.00 ± 440.83**</td>
</tr>
</tbody>
</table>

* values from 10 instruments – mean ± standard deviation
** values were not statistically different (p>0.05) as determined by one-way analysis of variance (ANOVA)

Table 2. Remaining contaminants after processing

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Cleaning time (sec)</th>
<th>Visible blood#</th>
<th>Hemastix positives</th>
<th>Hemastix values</th>
<th>Protein positives</th>
<th>Protein values</th>
<th>% protein removedd</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>90</td>
<td>2/15</td>
<td>15/15</td>
<td>3.3</td>
<td>13/15</td>
<td>15.53 ± 8.95</td>
<td>99.51</td>
</tr>
<tr>
<td>B</td>
<td>90</td>
<td>2/15</td>
<td>15/15</td>
<td>3.0</td>
<td>13/15</td>
<td>16.00 ± 9.89</td>
<td>99.52</td>
</tr>
<tr>
<td>C</td>
<td>90</td>
<td>0/15</td>
<td>15/15</td>
<td>3.3</td>
<td>13/15</td>
<td>16.33 ± 10.30</td>
<td>99.50</td>
</tr>
<tr>
<td>D</td>
<td>90</td>
<td>0/15</td>
<td>15/15</td>
<td>1.3*</td>
<td>6/15</td>
<td>4.93 ± 3.62</td>
<td>99.84</td>
</tr>
<tr>
<td>A</td>
<td>120</td>
<td>1/15</td>
<td>15/15</td>
<td>3.1</td>
<td>5/15</td>
<td>8.40 ± 14.88</td>
<td>99.73</td>
</tr>
<tr>
<td>B</td>
<td>120</td>
<td>3/15</td>
<td>15/15</td>
<td>3.3</td>
<td>6/15</td>
<td>4.80 ± 6.36</td>
<td>99.86</td>
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<tr>
<td>C</td>
<td>120</td>
<td>5/15</td>
<td>15/15</td>
<td>3.5</td>
<td>10/15</td>
<td>14.80 ± 16.44</td>
<td>99.55</td>
</tr>
<tr>
<td>D</td>
<td>120</td>
<td>2/15</td>
<td>15/15</td>
<td>2.3*</td>
<td>2/15</td>
<td>4.93 ± 3.62</td>
<td>99.84</td>
</tr>
<tr>
<td>A</td>
<td>300</td>
<td>0/15</td>
<td>15/15</td>
<td>2.3</td>
<td>3/15</td>
<td>2.93 ± 6.36</td>
<td>99.91</td>
</tr>
<tr>
<td>B</td>
<td>300</td>
<td>0/15</td>
<td>15/15</td>
<td>2.7</td>
<td>6/15</td>
<td>4.93 ± 6.88</td>
<td>99.85</td>
</tr>
<tr>
<td>C</td>
<td>300</td>
<td>0/15</td>
<td>15/15</td>
<td>2.7</td>
<td>7/15</td>
<td>7.13 ± 7.40</td>
<td>99.78</td>
</tr>
<tr>
<td>D</td>
<td>300</td>
<td>0/15</td>
<td>10/15</td>
<td>0.9*</td>
<td>2/15</td>
<td>1.73 ± 4.71</td>
<td>99.94</td>
</tr>
</tbody>
</table>

# Results based on three runs of five instruments
# Number positive over total number of instruments
* Qualitative readings of Trace, Small, Moderate and Large were converted to numerical values and the average presented (Trace = 1; Small = 2; Moderate = 3; Large = 4)
# Average protein detected from all 15 instruments ± standard deviation
d Based on the amount of protein present on control instruments (average: n = 15)
The difference of this value from the other three values at each time period is statistically significant (p = 0.001)

Add 3.0 mL of reagent E and mix 0.5% CuSO4 - 5H2O in deionized water