INTRODUCTION

Infection is one of the most important causes of joint implant failure. The polymerase chain reaction (PCR) is a sensitive way to test for bacteria, but most PCR procedures take too long to provide results during a revision arthroplasty operation. For example, we have described the sensitivity and specificity of real-time PCR combined with ultrasonication of retrieved implants to detect trace levels of bacteria within about 4 hours from the time of implant removal. It would be desirable to have the results of PCR testing available more rapidly.

Most PCR techniques require time-consuming extraction of DNA from the sample, but several studies have suggested that ultrasonication alone may yield adequate DNA for PCR analysis. The purpose of this study was to evaluate reducing the time required for PCR by ultrasonication without subsequent DNA extraction using an in vitro implant infection model.

MATERIALS AND METHODS

In vitro infection model - Twenty-five stainless steel plates (2 cm by 2 cm, 1 mm thickness) were used for the in vitro infection model. Biofilm formative S. aureus (ATCC 12600) was used for inoculation. Each stainless plate was inoculated with a 1 ul of 0.5 MacFarland units of bacteria in 10 ml of culture medium (TSB containing 2 % glucose) and incubated for 4 hours as an initial incubation. The plate was washed with PBS, placed in a fresh bag with 25 ml of PBS (Branson Ultrasonic Cleaner; Branson Ultrasonics, Danbury, CT). Sonicated solutions were collected and evaluated by real-time PCR directly.

Ultrasonication processing - Five groups of 5 plates each were used to determine the optimum duration of sonication (0, 1, 5, 10, 30 min). Each plate was sonicated in a sterile bag with 25 ml of PBS (Branson Ultrasonic Cleaner; Branson Ultrasonics, Danbury, CT). Sonicated solutions were collected and evaluated by real-time PCR directly without additional DNA extraction.

Real-Time PCR - S. aureus species specific primer and probe sets (Sa442) were used for quantitative real-time PCR using a LightCycler® (Roche). The PCR amplification mixture solutions were made according to the original reference.

RESULTS

Figure 1 shows an example of PCR results without DNA extraction. As this figure indicates, there were PCR negative samples in 0, 1, or 5 min. sonication groups. While all samples were PCR positive after 10 or 30 min. sonication. In all 25 samples, there were 4 PCR negative samples: 2 in the 0 min. sonication, 1 in 1 min. and 1 in 5 min. sonication groups (Table 1). Figure 2 shows the difference in PCR cycle number between no sonication and sonication (5 minutes) groups. There was a significant difference (P<0.05), indicating that without the DNA extraction step, sonication yielded more bacterial DNA than if sonication was omitted.

Table 1 - All PCR results for each sonication time.

<table>
<thead>
<tr>
<th>Sonication Time</th>
<th>PCR Negative</th>
<th>PCR Positive</th>
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</thead>
<tbody>
<tr>
<td>0 min.</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1 min.</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>5 min.</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>10 min.</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>30 min.</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

DISCUSSION

DNA extraction of some type is essential to test for bacterial DNA in tissue samples, and DNA extraction usually takes 2 or 3 hours to complete. Previous reports demonstrated the possibility of ultrasonication for releasing DNA without additional extraction methods for spore formative bacteria, and our results suggest that it may be possible to get PCR results without additional DNA extraction within one hour from implant removal, if 10 min. of sonication is applied.

In this study, the importance of sonication was confirmed in the samples without prior DNA extraction, but it is unclear if this effect is due to the release of DNA from bacteria, or to the dislodgement of biofilm (or both). Additional studies are also needed to compare the efficacy of sonication with other DNA extraction methods. In addition, clinical samples must be examined with this method to confirm its sensitivity. Nevertheless, we demonstrated the possibility of the direct application of real-time PCR, combined with ultrasonication for more rapid identification of infection around joint implants.

REFERENCES


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