THE EFFECT OF ULTRASONICATION ON THE DETECTION OF BACTERIA IN A BIOFILM

QUANTITATIVE REAL-TIME PCR AND CULTURE RESULTS USING AN IN VITRO IMPLANT INFECTION MODEL

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Introduction
The importance of bacterial biofilm associated with joint implants has been recognized widely and investigated. These bacteria are best detected after the biofilm has been disrupted by, for example, ultrasonication. Although ultrasonication enhances detection of adherent bacteria, it may kill the organisms, decreasing the sensitivity of conventional culture results. In addition, the optimum duration of ultrasonication to detect biofilm formative bacteria has not been investigated.

The aim of this study was to investigate the effect of ultrasonication on the viability of adherent bacteria, to compare the use of polymerase chain reaction (PCR) and culture to detect organisms after ultrasonication, and to determine the optimum duration of ultrasonication to maximize detection of bacteria adherent to a metal substrate.

Materials and Methods
<In vitro infection model> Twenty-five polished stainless steel plates (2 cm by 2 cm, 1 mm thickness) were used for an in vitro infection model. Each stainless plate was inoculated with 1 µl of 0.5 MacFarland standards of biofilm formative S. aureus (ATCC 12600) 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 once, placed in a fresh container with fresh medium, and incubated for 15 hours as a second incubation. Then the plate was washed twice with 25 ml of PBS to remove non-adherent bacteria from the implant surface.

<Ultrasonication processing> Five time durations of sonication were examined (0, 1, 5, 10, 30 minutes, five plates were examined for each time duration). Each plate was placed in a sterile bag with 25 ml of PBS, and sonicated (Branson Ultrasonic Cleaner; Branson Ultrasonics, Danbury, CT) at a frequency of 40 kHz for each time-duration. After sonication, an aliquot of the solution was collected and submitted for quantitative culture and real-time PCR.

<Quantitative culture> Each sonicated solution was cultured in duplicate with dilution of 1:1000 on BBL culture medium plate for 24 hours. Colony forming units (CFU) were counted for each plate and averaged for each time-duration group.

<DNA extraction> DNA extraction was performed using MagNA Pure LC Total Nucleic Acid Isolation Kit on a MagNA Pure instrument (Roche Diagnostics, Indianapolis, IN).

<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. Five micro liters of sample and 15 µl of master mix solution were used for each sample.

<Statistical analysis> One factor ANOVA was used for culture and PCR results. Turkey-Kramer test was performed for multiple comparison of each time group. The correlation between the culture and PCR results were evaluated using Pearson’s correlation coefficient test.

Results
<Quantitative culture> Figure 1-A shows the quantitative culture results. With no sonication (0 min.), the fewest bacteria grew, while the most bacteria grew after 1 min. of sonication. There were significant differences between 0 min. and 1 min., and also between 1 min. and 30 min. groups (P<0.05).

<Real-Time PCR> All 25 samples were PCR positive. The greatest number of bacterial DNA copies were detected following 1 min. of sonication, but with PCR, there was no statistical difference (P=0.35) among the 5 time groups (Figure 1-B). Figure 2 shows a correlation between culture and PCR results (P=0.0002).

Discussion
There have been several studies investigating bacteria and biofilm adherent to orthopaedic implants. Ultrasoundation may be the most effective and easy method to disrupt the biofilm, but ultrasonication may also kill bacteria. In this study, we confirmed that longer sonication times reduced bacterial viability, resulting in fewer viable colonies from conventional culture. PCR results were less affected by prolonged sonication than were culture results, because PCR detects bacterial nucleic acids independent of viability.

One limitation of this study is the nature of this implant infection model. A longer incubation duration might be associated with more biofilm and the nature of the substrate (stainless steel in our model) may also influence bacterial and biofilm adherence. Nevertheless, we documented significant differences in bacterial detection based on duration of ultrasound treatment and the type of bacterial assay. The combination of PCR and relatively brief ultrasonication appears to be a sensitive tool for the detection of bacteria adherent to implants; further studies are still needed to determine the clinical importance of these bacteria.

Acknowledgement
Financial support provided in part by Nippon Stryker K.K., Tokyo, Japan and by Stryker Orthopaedics, Mahwah, NJ.