Time-Dependent Effects of Chlorhexidine Soaks on Grossly Contaminated Bone

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Objective: The purpose of this study was to quantify the reduction in the bacterial burden of grossly contaminated bone segments using different chlorhexidine (CHL) solutions. We hypothesized that 4% CHL would be the most efficient decontaminate.

Methods: Fifty four bone segments were harvested from fresh frozen porcine legs. Each specimen was dropped onto a Mueller Hinton medium that was inoculated with *Staphylococcus aureus* (lux). These genetically engineered bacteria emit photons in propor tion to their number, allowing for quantification. The segments were retrieved after 5 seconds of exposure. Baseline imaging provided the initial bacterial load. An equal number of specimens were soaked in normal saline (NS), 2%CHL, or 4%CHL. Specimen reimaging was completed at the 5 , 10 , 20 , 30 , and 60 minute marks.

Results: The average bacterial count on the bone segments were 2.18×10^7 for NS, 2.31×10^7 for 2%CHL, and 2.00×10^7 for 4%CHL. The percent reduction in bacterial counts at the 5 , 10 , 20 , 30 , and 60 minute marks were NS: 0%, 0%, 0%, 29.84%, 72.23%; 2%CHL: 93.09%, 98.16%, 99.21%, 99.63%, 99.81%; 4%CHL: 94.32%, 97.60%, 99.25%, 99.63%, 99.82%. At all time intervals, there was a significant difference between the 2%CHL and 4%CHL groups compared with the NS group ($P < 0.0001$) and no difference between the 2%CHL and 4%CHL groups.

Conclusions: This study provides new data supporting the use of CHL to decontaminate grossly soiled bone segments. To maximize efficiency and decrease potential untoward effects, the authors recom mend 20 minute soaks using 2% CHL for contaminated bone segments.

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INTRODUCTION

Gross contamination of bone during an operative procedure can have devastating consequences. Although there are a multitude of graft options and a large number of allograft bone and soft tissue graft procedures performed in the United States,[1] there are some situations in which there is no good substitute for a critical piece of bone. In those situations, how to best cleanse the contaminated bone segment becomes a vitally important question.

Previous literature has shown positive culture rates between 58% and 96% when grafts were dropped onto the floor and cultured.[2–5] It has been demonstrated that even small increases in the initial bacterial inoculation within bone leads to a disproportionately large increase of bacterial colonization on allograft surfaces.[1] Additionally, bacteria that are present on reimplanted bone may expedite the process of biofilm formation.[1,6,7] Therefore, grossly contaminated bone segments should be decontaminated with effective methods before their reimplantation.

Although events in which bone segments become grossly contaminated are relatively rare,[8,9] the prevention of infection, in most cases, is a more important determinant of patient outcome than implanted bone segment viability.[10–12] It is therefore no surprise that other studies have recommended methods for removing bacterial contamination that have known or suspected deleterious effects on the cell viability of the treated bone segments.[10,13–16]

Although there are multiple methods that have been described in peer-reviewed literature for cleansing grossly contaminated bone, most of those methods have had minimal scientific evaluation.[13–22] Chlorhexidine soaks have become one of the more popular methods for decontaminating grossly soiled bone and has both basic science literature and case reports to support its use.[3,15,16,19,23–28] Manufacturer guidelines recommend that the contact time for chlorhexidine (CHL) be 2 minutes[29] for surgical skin preparations, but the authors are unaware of any guidelines for CHL used on bone. The purpose of this study is to describe the temporal relationship between the concentration of CHL soaks and the amount of bacteria present on grossly contaminated bone segments.

MATERIALS AND METHODS

Bone Segment Preparation

Sixteen fresh-frozen cadaveric porcine quartered limbs were obtained. From those limbs, the femur, tibia, and fibula were harvested, and the soft tissue was removed.

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The diaphysis of each tibia was cut into 1-cm thick samples, keeping each segments size as similar as possible. A total of 54 bone segments were used in the experiment.

Simulation of Gross Contamination of Bone Segments

Staphylococcus aureus is one of the species of bacteria known to be present on operating room floors and has a high incidence of surgical site infections.[5] For these reasons, *S. aureus* was selected for use in this study. The bacterial broth prepared for this investigation consisted of *S. aureus* (lux) (Xenogen 29, Caliper Life Science, Hopkinton, MA) with a concentration of 10^8 colony-forming units per milliliter on a Mueller–Hinton medium.

The diaphyseal bone samples were divided into 3 groups, each containing 18 bone segments. Each specimen was dropped from a height of 6 in. In doing so, we made sure that the flat, trabecular bone with the surrounding cortical rim contacted the Mueller–Hinton medium inoculated with the *S. aureus* (lux). The dropped bone segments were retrieved after being in contact with the *S. aureus* for 5 seconds. This method of contamination was used to simulate the act of dropping a piece of bone onto a nonsterile surface and retrieving it.

Quantification of the Bacterial Contamination

After the specimens were retrieved, they were placed onto a clean plate with the side that contacted the *S. aureus* facing up. The *S. aureus* are genetically engineered to emit photons in proportion to their number allowing for quantification with the IVIS100 imaging system (Xenogen Corp, Alameda, CA). This system uses an optical charge couple device camera to count photon emissions. This bacterial imaging technique has been previously described.[30,31] Imaging software (LIVINGIMAGE V. 2.12, Xenogen Corp, Alameda, CA, and IGOR V.4.02A, WaveMetrics, Lake Oswego, OR) was used to superimpose the photon count onto a gray-scale background image yielding the location and photon intensity. A standard size region of interest was placed around the bone segments on the image and from this region of interest the total photon count was taken, which was directly proportional to the bacteria number on the bone segments.

After baseline imaging was obtained, the segments were soaked in 1 of 3 solutions. Group 1, which served as the control group, was soaked in 1 L of normal saline (NS), group 2 in 2%CHL, and group 3 in 4%CHL. Each bone segment was removed from its solution and reimaged after 5, 10, 20, 30, and 60 minutes. All of the bone segments were reimaged in an identical manner and position as that used to obtain the baseline imaging. This methodology allowed for a direct comparison of baseline bacterial load on each segment to each subsequent image at the aforementioned time interval, using repeated measures.

Statistical Analyses

Photon counts at each time point were compared with the baseline photon counts for each bone segment. All data were analyzed using 2-way analysis of variance with repeated measures and the Tukey–Kramer adjustment for multiple

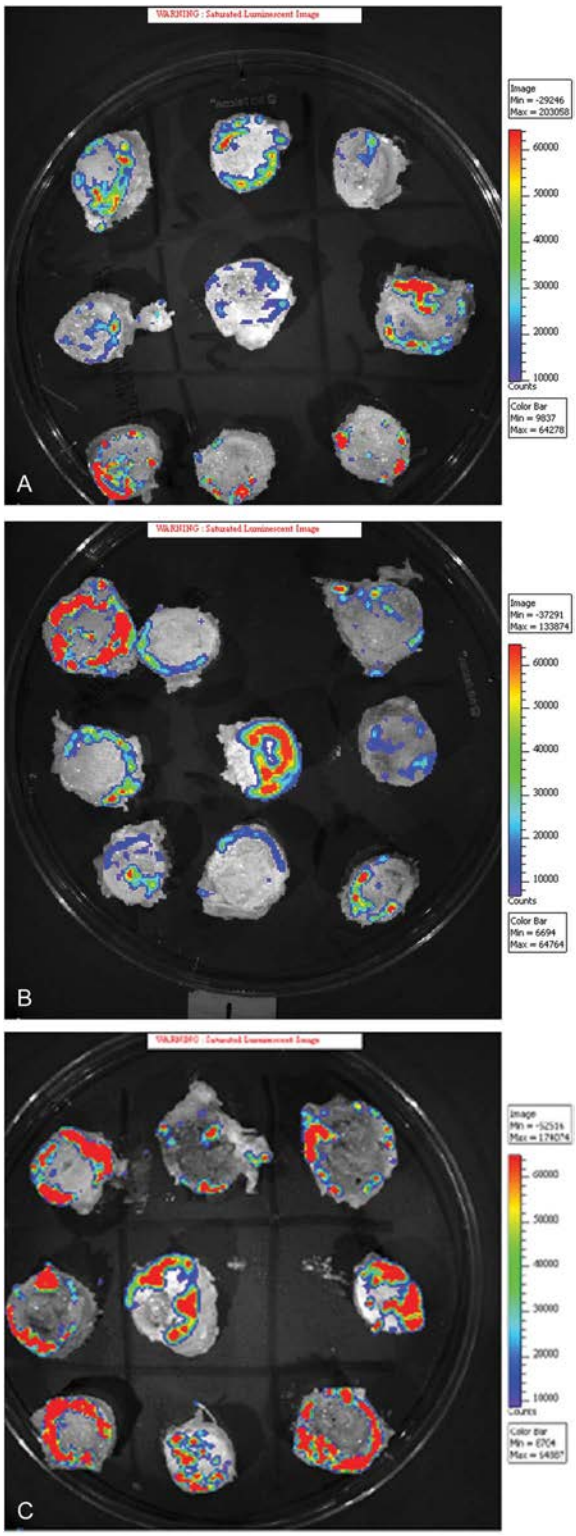

FIGURE 1. A C, Baseline imaging of bone segments soaked before being soaked in NS (top left), 2% CHL (top right), and 4% CHL (bottom right).

comparisons using SAS statistical software (SAS Institute, Cary, NC) with significance set at $P < 0.05$. All values are reported as average ± SEM.

RESULTS

The average baseline bacterial count was $2.18 \times 10^7 \pm 3.35 \times 10^6$ for the NS group, $2.31 \times 10^7 \pm 4.12 \times 10^6$ for the 2%CHL group, and $2.00 \times 10^7 \pm 3.55 \times 10^6$ for the 4%CHL group (Figs. 1A–C). There was no decrease in bacteria until 30 minutes for the NS group. Both the 2%CHG and 4%CHL groups demonstrated a rapid decrease in bacteria at 5 minutes, and there was a small decrement of bacteria at each of the other time periods (Table 1, Figs. 2A–C and 3).

At all time intervals, the difference between the 2% and 4% CHL groups compared with the NS group was found to be significant ($P < 0.0001$). The largest difference between the 2% and 4% CHL groups was seen early at 5 minutes, but this difference was not significant ($P < 0.9984$). There was no difference between the 2% and 4% CHL groups at all time points.

DISCUSSION

The gross contamination of a critical portion of bone is a scenario that orthopedic surgeons may encounter at some point in their careers.[5,8] The potential complications associated with using a soiled piece of bone are severe and must be weighed against the costs of discarding the segment of bone altogether, especially as we move toward an era of healthcare cost control. When the grossly contaminated segment is periarticular or when allograft is unavailable, there may be no option of discarding the soiled segment of bone. In those situations, the surgeon is forced to cleanse and replant the specimen. This study provides basic science evidence that soaking a grossly contaminated bone segment in 2% or 4% CHL for as little as 20 minutes removes >99% of the initial *S. aureus* burden.

Previous literature that has focused on culturing soft tissues and case studies has supported the use of CHL for decontamination of soft tissue grafts and bone segments.[3,15,19,23] The results of this study add further support for its use as 4% CHL decreased the bacterial load on the grossly contaminated pieces of bone by 94.32% at 5 minutes, 99.26% at 20 minutes, and 99.82% at 60 minutes. Soaks in 2% CHL had similar results with a 93.09% reduction in bacterial load at 5 minutes, 99.31% at 20 minutes, and 99.81% at

TABLE 1. Average Photon Count on Bone Segments Per Group

Time (min)	NS	2% CHL	4% CHL
0	2.18×10^7	2.31×10^7	2.00×10^7
5	3.34×10^7	1.45×10^6	6.62×10^5
10	2.96×10^7	3.43×10^5	2.72×10^5
20	1.92×10^7	1.31×10^5	7.78×10^4
30	1.27×10^7	6.73×10^4	3.83×10^4
60	6.21×10^6	3.44×10^4	2.08×10^4

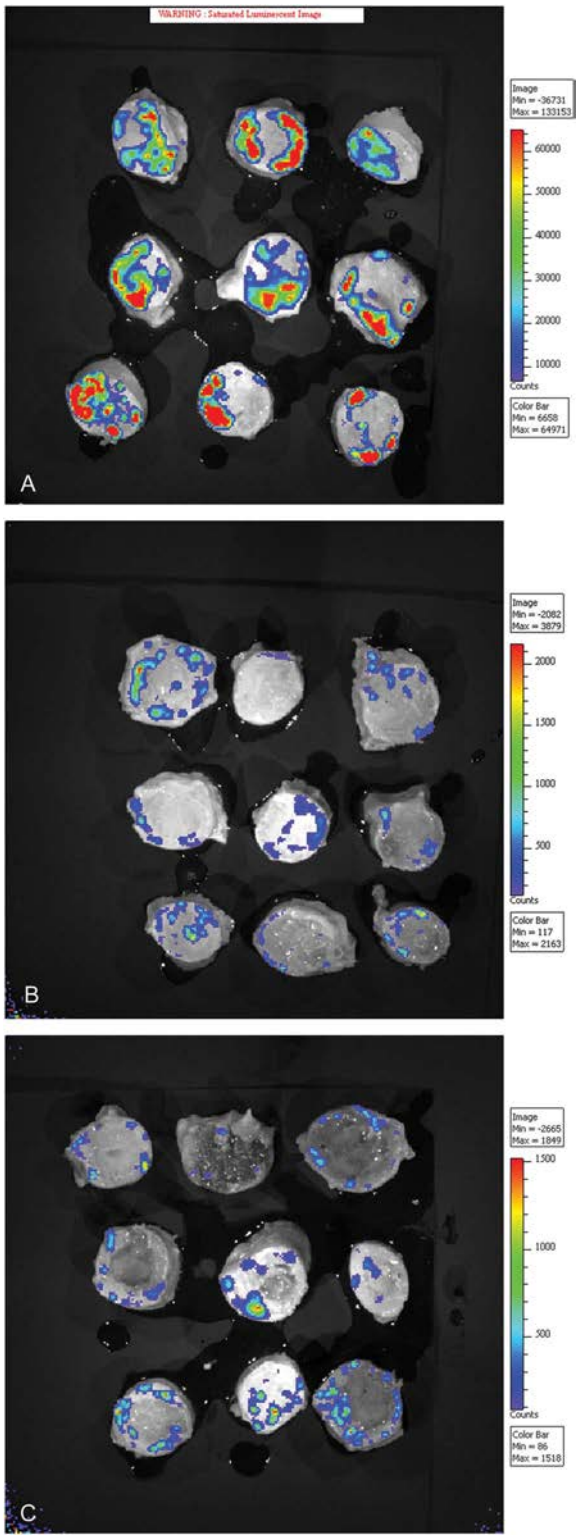

FIGURE 2. A C, Imaging of bone segments after being soaked in NS (top left), 2% CHL (top right), and 4% CHL (bottom right), for 20 minutes.

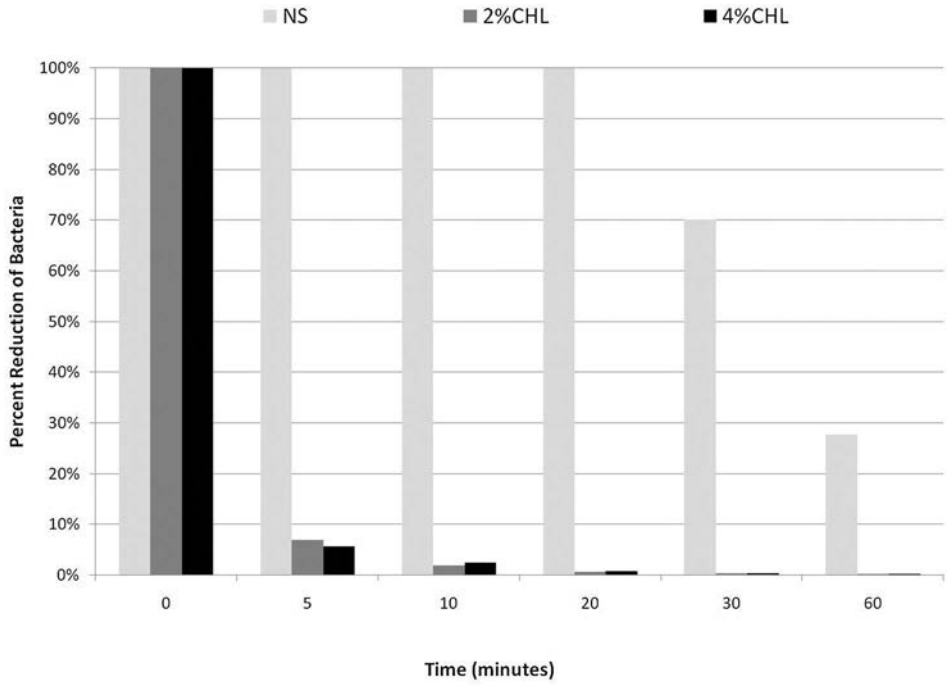

FIGURE 3. Average percentage of bacteria remaining on bone segments for each solution.

60 minutes. These data demonstrate the effectiveness of both the 2% and 4% CHL solutions in decreasing the bacterial load while demonstrating that the additional decrease in bacterial load gained by letting the bone segments soak for >20 minutes was minimal.

Two recent articles have examined how to best decontaminate osteochondral bone segments.[5,32] One of the potential flaws of these studies is that their data relied on culture results. In the study by Bruce *et al*,[5] while swabbing the operating room floor produced a positive culture in 100% of the samples, osteochondral fragments that were dropped onto the operating room floor showed a positive culture only 70% of the time, highlighting the potential lack of sensitivity cultures may have in detecting contaminated bone segments. Similar problems with the sensitivity of cultures in detecting contamination were found in the article of Bauer et al[32] where only 4 out of 10 swabs of the operating room floor grew positive cultures. Both studies most likely do not support the notion that the operating room floor that they were culturing was not contaminated but that the existing contamination on the floor was not captured by the cultures. This study avoids the possible biases associated with culture results by using bioluminescent bacteria that emitted photons in direct proportion to their metabolic activity. This approach allows for repeated measures of bacterial contamination and enabled the calculation of percent reduction in bacterial load on the bone segments.

The plateau in percent decreased in bacterial load after 20 minutes of soaking time found in our experiment provides evidence of how long grossly contaminated segments should be cleansed. Many studies have utilized different time lengths for CHL soaks.[3,15,19,23] This study suggests that 20 minutes is an adequate amount of time to soak a grossly contaminated segment of bone. This amount of time produced a decrease in bacterial quantity of 99.32% and 99.26% for the 2% and 4% CHL solutions, respectively. Decreasing unnecessary soaking time in CHL for decontamination is important as prolonged soaks can potentiate the negative effects of the CHL on the native tissue or bone specimen[23,25] while also increasing the operative costs[23] and potential anesthetic complications associated with longer operative case times.

Previous studies have also recommended against using CHL as a decontaminate because of its effects on cell viability after use.[5,32] Chlorhexidine has been shown to have many detrimental effects on native tissue,[2,8,25] and it has been suggested that CHL may impair osteoclastic and osteoblastic function at concentrations as low as 1%.[20] However, the studies demonstrating these effects, were completed in vitro where the normal biologic milieu to support cell growth is altered. In contrast, several in vivo case studies do show that the cellular effects of decontamination with CHL may not have a detrimental effect on clinical outcome or bone segment viability.[15,16] This study showed that 2% CHL was essentially just as effective as 4% CHL at decreasing bacterial load, and its use in place of 4% CHL may help decrease the potential damage CHL may have on native tissue.

This study has several limitations. Although it was completed in vitro and the translation of any in vitro evaluation to in vivo conditions can be problematic and additional preclinical and clinical evaluations are needed. The study examined the effects of 1 *S. aureus* strain and did not examine the effects of the CHL soaks on gram-negative or mixed groups of bacteria, both of which are likely to be encountered in the clinical setting. Also, we do acknowledge that the *S. aureus* (lux) on the Mueller–Hinton plates used to

contaminate the bone may not have been homogenous and some areas may have had higher concentrations of *S. aureus* (lux) than others. Using repeated imaging of the bioluminescent bacteria allowed for all the data to be analyzed with repeated measures for each bone segment and lessen the potential experimental bias. Also, the use of bioluminescent bacteria allowed for the effect of each treatment concentration and exposure time to be measured.

CONCLUSIONS

By using repeated measures to quantify the percent reduction in contaminating bacteria on bone, these study findings provide supportive data that has not previously been reported. This study provides a measure of how quickly and effective 2% and 4% CHL decreases the bacterial load on soiled bone. Because of the reported detrimental effects that CHL has on cell viability, we would recommend based on this in vitro evaluation a 2% CHL solution soak for 20 minutes for contaminated bone to potentially minimize the reported cell viability concerns. This study needs to be expanded to other bacterial types and strains and be evaluated in preclinical and clinical models. The reported data may contribute to the development of an evidence-based standardized protocol in the future for the decontamination of grossly contaminated bone.

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