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Are Dropped Osteoarticular Bone Fragments Safely Reimplantable in Vivo?

By Benjamin Bruce, MD, Shahin Sheibani-Rad, MD, Deborah Appleyard, MD, Ryan P. Calfee, MD, Steven E. Reinert, MS, Kimberle C. Chapin, MD, and Christopher W. DiGiovanni, MD

Investigation performed at the Department of Orthopaedic Surgery, Warren Alpert School of Medicine at Brown University, Providence, Rhode Island

Background: There are limited data detailing the appropriate management of nondisposable autologous osteoarticular fragments that have been contaminated by the operating room floor. The goal of the present study was to perform a comprehensive, three-phase investigation to establish an appropriate intraoperative algorithm for the management of the acutely contaminated, but nondisposable, autologous osteoarticular bone fragment.

Methods: Phase I of the study was performed to quantify the rate of contamination and microbial profile of human osteoarticular fragments that were dropped onto the operating room floor (n = 162). Phase II was performed to assess the feasibility and optimal means of decontaminating 340 similar fragments that underwent controlled contamination with bacteria that were identified in Phase I; decontamination was performed with use of cleansing agents that are routinely available in an operating room. Phase III was performed to assess the effect of each decontamination process on fragment chondrocyte viability through histologic evaluation.

Results: The contamination rate in Phase I was 70%. Coagulase-negative Staphylococcus was the most commonly cultured organism. In Phase II, varying exposure time to the chemical agents did not make a significant difference in decontamination rates. Mechanical scrubbing was superior to mechanical saline solution lavage (zero of fifty-six cultures compared with twenty of fifty-six cultures were positive for coagulase-negative Staphylococcus; $p < 0.001$). As a whole, bactericidal agents were found to be more effective decontaminating agents than normal saline solution. Povidone-iodine and 4% chlorhexidine gluconate were the most effective decontaminating agents, with none of the twenty-eight specimens that were decontaminated with each agent demonstrating positive growth on culture. Phase III demonstrated that the groups that were treated with normal saline solution and povidone-iodine retained the greatest number of live cells and the least number of dead cells. Mechanical scrubbing significantly decreased chondrocyte viability as compared with a normal saline solution wash ($p < 0.05$).

Conclusions: The majority of osteochondral fragments that contact the operating room floor produce positive bacterial cultures. Five minutes of cleansing with a 10% povidone-iodine solution followed by a normal saline solution rinse appears to provide the optimal balance between effective decontamination and cellular toxicity for dropped autologous bone in the operative setting.

Clinical Relevance: This study provides guidance on treatment of dropped osteoarticular fragments during surgery in order to decrease the rate of contamination and infection.

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A commentary by Benjamin K. Potter, MD, and Jonathan Agner Forsberg, MD, is available at www.jbjs.org/commentary and is linked to the online version of this article.

The management of inadvertent intraoperative contamination of a nondisposable autologous osteoarticular bone fragment poses a challenging dilemma to the orthopaedic surgeon. The operative team must weigh the risk of an infectious complication following reimplantation against that of discarding a potentially critical osteochondral host fragment. Despite the rarity of this event, at least one in four orthopaedic surgeons are likely to encounter this situation during their career[1,2]. The most commonly reported mechanism is accidentally dropping an osteochondral fragment onto the floor[3]. Previously, up to 58% of dropped grafts have been shown to grow positive cultures[4]. Contaminated osteoarticular fragments confront the surgeon with a unique problem as these pieces often cannot be discarded because of their unique shape and composition. At this time, the English-language literature does not provide a consensus decontamination algorithm to guide the surgeon when faced with this situation. The existing literature on the management of an autograft that is accidently contaminated during an operation has primarily focused on either the soft tissue involved with anterior cruciate ligament reconstruction or materials such as allograft, cadaveric bone, or even xenograft. The available published data regarding the optimal management of these contaminated specimens remain limited and are often conflicting[5-9]. Previously studied decontamination techniques have included mechanical scrubbing, serial dilution, exposure to various antibacterial agents, and autoclaving[4,8,10-14]. Previous studies have been limited as they have not tested osteoarticular fragments (which have a different contamination risk when compared with bone-tendon grafts), have not focused on the range of bacteria likely encountered on the operating room floor, and have not rigorously tested the impact of decontamination on cell viability[12,14-17].

The present study was designed as a three-phase investigation to identify a readily employable, scientifically justified, intraoperative management scheme for the surgeon who is faced with a contaminated but nondisposable autologous osteoarticular bone fragment.

We hypothesized that >50% of fresh osteoarticular bone fragments would demonstrate positive growth on culture following contact with the operating room floor and that the primary contaminant would be coagulase-negative Staphylococcus. Furthermore, we hypothesized there would be significant differences in decontamination rates between the chosen decontamination agents. We also hypothesized there would be a significant difference between the techniques utilized (specifically, that a ten-minute bactericidal bath would be superior to a five-minute bactericidal bath and that mechanical scrubbing would be superior to rinsing). Finally, we hypothesized that the effectiveness of each decontamination technique would be inversely related to post-treatment cartilage cell viability.

Materials and Methods

Institutional review board approval was obtained prior to the commencement of the present study. Phase I of the study was performed to quantify the rate of contamination and the microbial profile of human osteoarticular fragments that were dropped onto the operating room floor. Fourteen direct swabs of the operating room floors used during the investigation were analyzed to reasonably characterize the operating room floor flora. Fresh osteoarticular bone specimens were sterilely recovered during routine primary knee arthroplasties. Each specimen was carefully cut into 1 to 2-cm^3 osteoarticular fragments, with attention to meticulous sterile technique. A total of 162 fragments were obtained from six patients. All osteoarticular fragments were stripped of surrounding soft tissues. Each fragment was then separately dropped onto the operating room floor at the conclusion of the arthroplasty, adjacent to the operative table. No changes to the floor-cleaning protocol had been requested prior to the operation. Each of these fragments was permitted to rest on the operating room floor for a total of thirty seconds before being collected for tissue culture with sterile forceps. Each retrieved specimen was immediately transported to the microbiology laboratory, where it was rolled onto aerobic and anaerobic blood agar plates for at least twenty seconds to ensure that all sides contacted the culture plate. Each plate was then incubated in 5% CO_2 for forty-eight hours for aerobic growth and for seven days in sealed jars for anaerobic growth. If the plate showed growth, the organisms were fully identified by means of standard clinical microbiological procedure. The three most common bacterial organisms identified in Phase I were determined to be appropriate candidates for further testing in Phase II.

Phase II of the study was then performed to assess the optimal means of decontaminating an osteoarticular fragment that had been exposed to the most common organisms cultured in Phase I. All test specimens underwent a two-part sequence involving chemical decontamination followed by mechanical decontamination. The chemical decontamination agents that were tested included routine preoperative scrubs that are used in many operating rooms, specifically, 10% povidone-iodine (Betadine), 4% chlorhexidine (Hibiclens), 70% isopropyl alcohol/2% chlorhexidine gluconate (Chloraprep), and 0.9% normal saline solution. Mechanical decontamination was performed via either a one-minute lavage with normal saline performed with a bulb syringe ("rinse") or a one-minute physical scrub performed with a bristled sponge ("scrub").

For this phase of the investigation, a total of 340 fresh osteochondral fragments were recovered in a fashion similar to that described in Phase I. All bone fragments were placed into separate sterile cups and were inoculated with 100 μL (450 to 480 colony-forming units) of microorganisms, a bacterial load found to be present on operating room floors[18]. Based on the most commonly identified bacteria in Phase I, 140 specimens were inoculated with coagulase-negative Staphylococcus (the most often identified organism), 100 were inoculated with Bacillus, and 100 were inoculated with Corynebacterium. All fragments were immersed for five minutes.

Of the 140 coagulase-negative Staphylococcus fragments, twenty-eight were set aside as the control group and underwent no further intervention and the remaining 112 fragments were divided equally into four decontamination test groups of twenty-eight specimens each. Each specimen was individually placed into separate 20-mL baths containing the specified decontamination agent. Within each test group, fourteen specimens were bathed in the assigned solution for five minutes and the other fourteen were bathed for ten minutes. On completion of the immersions, half (seven) of the fragments in each subgroup were mechanically brush-scrubbed under sterile conditions for one minute ("scrub") and the other half were sterilely lavaged for one minute with normal saline solution with use of a bulb syringe ("rinse"). All 140 test fragments were then rolled on blood agar plates for at least twenty seconds to ensure that each side was exposed to the culture media. Each plate was then incubated in CO_2 for seven days and was examined daily for growth (Figs. 1 and 2).

The Bacillus and Corynebacterium-exposed fragments were similarly treated. Of the 100 fragments in each group (Bacillus or Corynebacterium), twenty were set aside as the control group and underwent no further processing. The remaining eighty fragments in each group were placed individually into separate 20-mL baths of the chemical decontamination agents. Each test subgroup subsequently underwent identical treatments to those of the coagulase-negative Staphylococcus group described above.

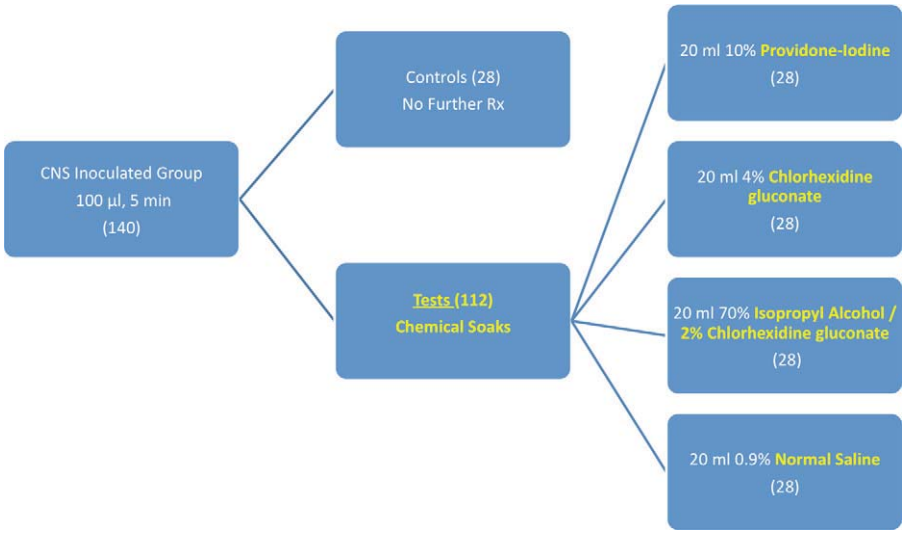

Fig. 1

Diagram illustrating the distribution of specimens in the coagulase-negative Staphylococcus (CNS) arm in Phase II. The 140 specimens were split into five equal groups of twenty-eight specimens to be treated with povidone-iodine, 4% chlorhexidine gluconate, 70% isopropyl alcohol with 2% chlorhexidine gluconate, or normal saline solution as well as a control group that received no further treatment (Rx). The number of fragments in each group is shown in parentheses. The Bacillus and Corynebacterium arms were similar, except that a total of 100 fragments were used in each arm.

Phase III was performed to assess the effect of each decontamination process on chondrocyte viability within the osteoarticular fragments. A total of 101 similarly sized (1 to 2-cm^3) bone fragments were again recovered during primary knee arthroplasties, as previously described, and were taken to the laboratory within fifteen minutes of harvest. All fragments were subjected to a five-minute chemical decontamination bath (based on the results in Phase II), followed by either a one-minute saline solution rinse or one-minute mechanical scrub. Twenty-four fragments were exposed to povidone-iodine

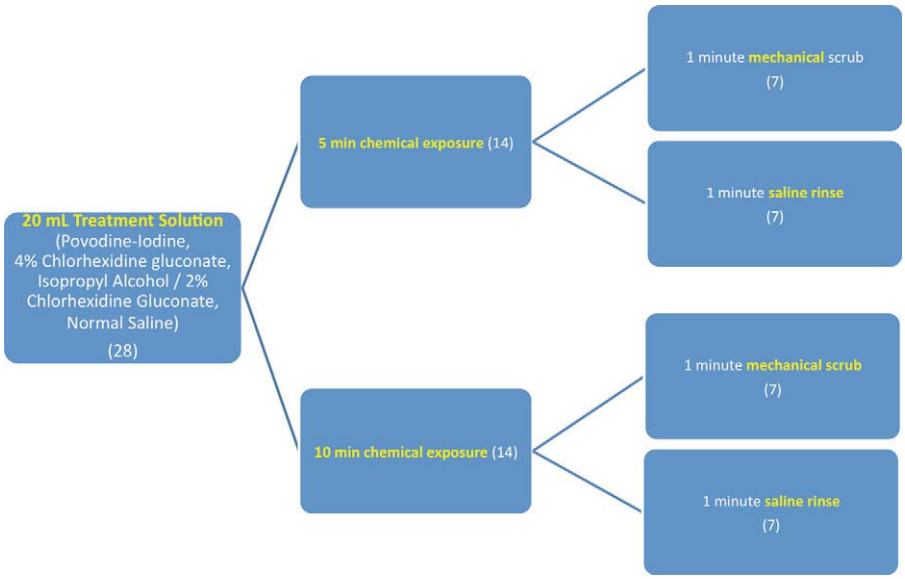

Fig. 2

Diagram illustrating the treatment protocol for each of the groups in the coagulase-negative Staphylococcus (CNS) arm in Phase II. Each specimen was treated in a chemical bath for either five or ten minutes. Next, each specimen underwent mechanical decontamination with either a 150-mL normal saline solution rinse or one-minute mechanical scrub. Finally, each fragment was rolled onto culture plates for twenty seconds and was incubated in CO_2 for seven days. The number of fragments in each group is shown in parentheses.

followed by a saline solution rinse (twelve) or a mechanical scrub (twelve), twenty-one fragments were exposed to 4% chlorhexidine followed by a saline solution rinse (eleven) or a mechanical scrub (ten), twenty fragments were exposed to isopropyl alcohol/2% chlorhexidine followed by a saline solution rinse (ten) or a mechanical scrub (ten), twenty-three fragments were exposed to a saline solution rinse (twelve) or a mechanical scrub (eleven), and thirteen control fragments received no treatment. Each fragment was subsequently stained with a ten-minute immersion in 40 μg/mL of propidium iodide and 1-μM fluorescein diacetate. Following treatment, specimens were taken to the confocal microscope for analysis within thirty minutes. The specimens were initially screened for cellular homogeneity to ensure that they were grossly similar. Confocal microscopic examination was performed to assess the percentage of retained viability of the chondrocytes. All cells were electronically counted. Manual review of these cell counts was performed to ensure the accuracy of the software. The percentage of live cells was determined by dividing the mean number of live cells per specimen by the total number of live and dead cells per specimen.

Images were acquired with a Nikon C1si confocal microscope (Nikon, Melville, New York) using 481 and 561-nm diode lasers. A minimum of three fields, and as many as seven fields, were captured per specimen. More fields were captured if time permitted between the processing of specimens. For each field examined, images were made every 0.5 μm in the Z axis for a 20-μm thickness, generating approximately forty images, so that not only surface cells were included in the count. These images were then collapsed on top of each other, forming one projection image. Cell counts were performed on the projection image, ensuring that the same cell was not counted twice, with use of iVision software (version 4.0.10; BioVision Technologies, Exton, Pennsylvania).

Statistical Analysis

Chi-square analysis was used to test for relationships between culture growth and bone-fragment treatment type. To test the relationships between live-cell and dead-cell counts among five cleansing agents, we employed the Kruskal-Wallis equality-of-populations rank test. To assess differences between mechanical scrubbing and saline solution rinsing, the Wilcoxon rank-sum test was employed. An alpha probability of 0.05 was used as the threshold for significance in two-tailed comparisons. Confidence intervals were calculated

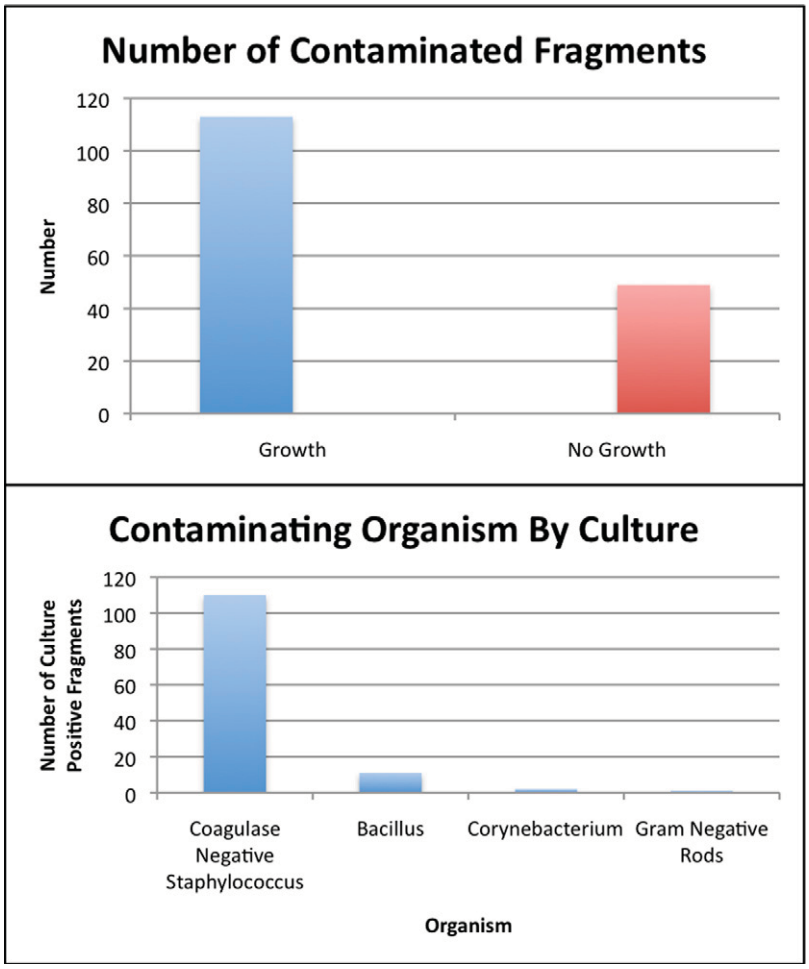

Fig. 3

Bar graphs showing the results in Phase I. The top figure shows that 113 (70%) of 162 grafts that were dropped on the floor had positive cultures. The bottom figure shows that 110 (97%) of the 113 cultures produced coagulase-negative Staphylococcus, with Bacillus species representing the second most common organism. All direct swabs of the operating room floor demonstrated growth of coagulase-negative Staphylococcus (data not shown).

TABLE I Culture Positivity After Treatment*

Agent	5-Minute Bath		10-Minute Bath	
	Rinse	Scrub	Rinse	Scrub
Saline solution	7/7	0/7	7/7	0/7
Iodine	0/7	0/7	0/7	0/7
Isopropyl alcohol + chlorhexidine	4/7	0/7	2/7	0/7
Chlorhexidine	0/7	0/7	0/7	0/7

*The data are given as the number of fragments.

for the percentage of live cells for each treatment group. All statistical analyses were performed with Stata software (version 10; Stata, College Station, Texas).

Source of Funding

Funding for this study was obtained from the Department of Orthopaedics of the Warren Alpert School of Medicine. The funds were used to purchase materials and capital equipment only, including reagents, disposable culture material, and an incubator. No funds were used for salaries.

Results

Phase I

All fourteen operating room floor swabs (100%) demonstrated growth of coagulase-negative Staphylococcus. Six swabs (43%) demonstrated growth of Bacillus species. Other species that were identified included one colony each of Micrococcus species, *Pseudomonas aeruginosa, Acinetobacter haemolyticus, Escherichia coli,* Pantoae species, *Serratia odorifera,* and *Staphylococcus aureus.*

One hundred and thirteen (70%) of the 162 fragments that were dropped on the floor for thirty seconds demonstrated bacterial growth (Fig. 3, *top*). Coagulase-negative Staphylococcus grew on culture of 110 (97%) of the 113 fragments, Bacillus grew on culture of eleven fragments (10%), Corynebacterium grew on culture of two fragments (1.7%), and gram-negative rods grew on culture of one fragment (<1%) (Fig. 3, *bottom*). More than one species grew on culture of eleven (7%) of the 162 fragments.

Phase II

Coagulase-Negative Staphylococcus

As expected, 100% of the twenty-eight untreated (control) specimens inoculated with coagulase-negative Staphylococcus demonstrated positive cultures. The control specimens demonstrated a mean of ninety-eight colony-forming units. Of the fourteen specimens that were decontaminated with use of a normal saline solution bath, 100% demonstrated positive cultures if no mechanical scrub was performed (Table I). An average of twenty-one colony-forming units grew following the five-minute bath and an average of eleven colony-forming units grew following the ten-minute bath. The number of colony-forming units was significantly higher for the untreated (control) specimens than for the specimens that were treated with saline solution for either five or ten minutes ($p < 0.001$). In the test groups, specimens that were decontaminated with either povidone-iodine or 4% chlorhexidine demonstrated no growth, regardless of the duration of exposure or the means of mechanical cleansing (scrub or lavage). In specimens that were sterilized with 70% isopropyl alcohol with 2% chlorhexidine gluconate, dose-dependent growth was detected if no mechanical brush-scrub was performed, with four of seven fragments demonstrating a positive culture after a five-minute bath and two of seven fragments demonstrating positive cultures after a ten-minute bath ($p > 0.05$). Alternatively, twenty (36%) of fifty-six specimens that underwent a saline solution lavage exhibited growth, whereas none of fifty-six brush-scrubbed specimens demonstrated a positive culture (chi square = 24.3; $p < 0.001$). Fourteen (50%) of the twenty-eight specimens that were treated with saline solution and six (21%) of the twenty-eight specimens that were treated with 70% isopropyl alcohol with 2% chlorhexidine demonstrated growth, whereas none of the specimens

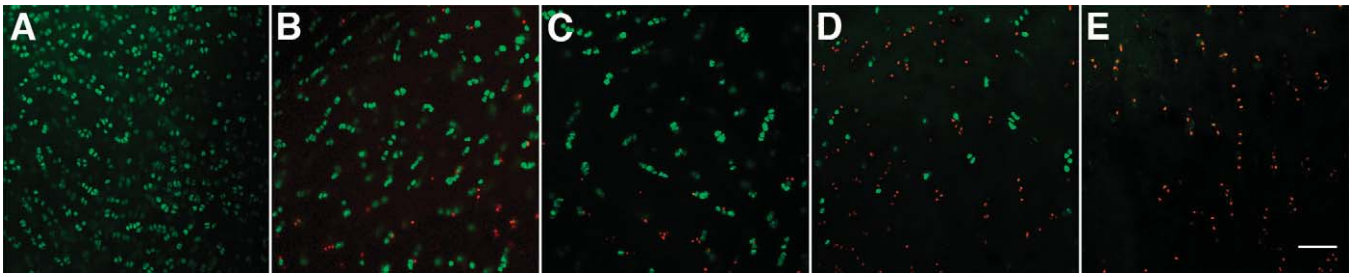

Fig. 4

Representative fluorescent photomicrographs of stained cartilage cells from specimens in Phase III, made with use of a confocal microscope. After undergoing chemical and mechanical sterilizing processes identical to those in Phase I, each fragment was subsequently stained with a ten-minute immersion in 40 μg/mL of propidium iodide and 1-μM fluorescein diacetate. Fluorescein diacetate will fluoresce green in viable cells, whereas propidium iodide, excluded by viable cells, will bind the DNA of dead or dying cells and fluoresce red. Each field is shown at 20× magnification. *A:* No treatment (control). *B:* Saline solution wash, saline solution lavage, no scrub. *C:* Iodine wash, saline solution lavage, no scrub. *D:* Isopropyl alcohol wash, saline solution lavage, no scrub. *E:* Chlorhexidine wash, saline solution lavage, no scrub.

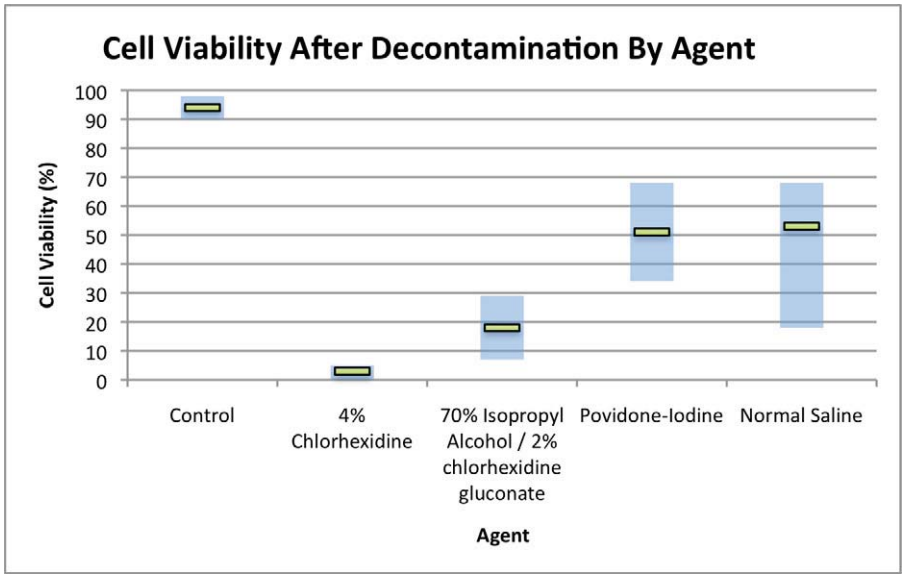

Fig. 5

Box plot illustrating cell viability after decontamination. Chondrocyte viability was measured in osteochondral fragments after the specified treatments. Specimens treated with saline solution or 10% povidone-iodine had the highest cell viability. Four-percent chlorhexidine was most toxic to chondrocytes. The mean cell viability is indicated by the green bars, with the associated 95% confidence interval represented by the blue bar.

that were treated with either povidone-iodine or 4% chlorhexidine demonstrated growth (chi square = 32.1; $p < 0.0001$). No significant difference was detected in any of the groups on the basis of the duration of bath treatment (five or ten minutes) ($p = 0.62$) (Table I).

Bacillus

One hundred percent of the control specimens that were inoculated with Bacillus demonstrated positive cultures. No specimen that was sterilized with povidone-iodine, 4% chlorhexidine, or isopropyl alcohol/2% chlorhexidine gluconate, regardless of duration of exposure or type of mechanical treatment (scrub or saline solution rinse), demonstrated growth. In this group, the single culture-positive fragment following treatment had been exposed to the five-minute normal saline solution bath without mechanical scrub. Otherwise, all fragments demonstrated no growth, independent of agent, exposure duration, mechanical scrub, or normal saline solution rinse.

Corynebacterium

Only nine (45%) of the twenty control specimens inoculated with Corynebacterium exhibited positive cultures. No growth was noted on any specimen, regardless of exposure or decontamination method.

Phase III

In the control group of untreated osteoarticular fragments (n = 13), 94% of the cells were viable (95% confidence interval, 90% to 98%). Overall, the control group demonstrated increased cell viability relative to the group of specimens that underwent decontamination treatment (94% compared with 32%; $p < 0.0001$). Four-percent chlorhexidine was the agent that was most toxic to articular cells, generating a cell viability of 3% (95% confidence interval, 0% to 5%) (n = 21). Specimens that were treated with 70% isopropyl alcohol with 2% chlorhexidine exhibited a cell viability of 18% (95% confidence interval, 7% to 29%) (n = 20). Specimens that were treated with povidone-iodine demonstrated a cell viability of 51% (95% confidence interval, 34% to 68%) (n = 24). Of the treatment groups, saline solution demonstrated the highest cell viability of 53% (95% confidence interval, 18% to 68%) (n = 23) (Figs. 4 and 5). A one-minute mechanical scrub, as compared with a one-minute normal saline solution bulb syringe lavage, significantly negatively affected cell viability. Specimens that were scrubbed demonstrated a cell viability of 18% (95% confidence interval, 10% to 25%), whereas specimens that were lavaged demonstrated a cell viability of 60% (95% confidence interval, 49% to 71%).

Discussion

Several important findings can be gleaned from this study and employed to generate more accurate and useful conclusions regarding an optimal algorithm following the accidental intraoperative contamination of osteoarticular grafts. In Phase I, 70% of osteoarticular fragments that were dropped onto an operating room floor produced positive bacterial cultures, predominantly coagulase-negative Staphylococcus. Hirn et al. contaminated sixty femoral head specimens by rubbing them onto the operating room floor and also found coagulase-negative Staphylococcus and Bacillus species as the

most prevalent organisms[19]. Our data are consistent with other previously reported operating room microbial profiles[18,20], suggesting that our results are applicable to other operating room environments. Neither methicillin-resistant nor pan-sensitive *Staphylococcus aureus* were identified on any of our cultured osteoarticular fragments.

The two most common organisms identified from direct swabs of the operating room floor were the same as those found on dropped osteoarticular fragments: coagulase-negative Staphylococcus and Bacillus species. While other organisms were identified by direct swab, they were not grown on culture of the contaminated specimens. This finding suggests that these other organisms may be less adherent or less viable on osteochondral fragments compared with cotton swabs. Overall, however, the collective data suggest that individual institutions could use direct swabs of the operating room floor to largely predict organisms most likely to contaminate bone.

In Phase II, each of our specimens was inoculated with a bacterial load approximating 450 colony-forming units, as typically found on an operating room floor[18]. As expected, untreated cells demonstrated significantly higher numbers of colony-forming units relative to specimens treated with just saline solution lavage ($p < 0.001$). Our data indicate that the chemical agents significantly reduced contamination compared with normal saline solution. Four-percent chlorhexidine and povidone-iodine appeared to be the most effective agents without consideration of the effects on cell viability. In fact, no positive bacterial culture was produced by a fragment treated with either agent, independent of length of chemical exposure time or type of mechanical sterilization. In one previous study, 4% chlorhexidine demonstrated effectiveness in completely decontaminating grafts, but this was with a ten to twelve-minute bath in conjunction with power irrigation[15]. Several other previous studies have shown that povidone-iodine is less effective for cleansing autografts contaminated with *Staphylococcus aureus* or *Pseudomonas aeruginosa.* However, neither we nor other authors have found these species to colonize contaminated bone that has been dropped during surgery[4,9]. Our results are consistent with the finding, reported by Soyer et al., that a five-minute exposure to iodine can decontaminate osteoarticular fragments[16]. Notably, Soyer et al. found that contaminated specimens required a minimum of five minutes to achieve decontamination. Our study did not assess a shorter exposure time, but, given the lack of any statistical difference between the five-minute and ten-minute exposures, shorter exposures may provide adequate decontamination given the additional mechanical protocol we employed. Contrary to our initial hypothesis, isopropyl alcohol/2% chlorhexidine did not achieve negative bacterial culture in all fragments. Coagulase-negative Staphylococcus remained less susceptible to isopropyl alcohol/2% chlorhexidine than either Bacillus or Corynebacterium, which may be explained by the fragility of the latter organisms[21-23]. In contrast, Burd et al. noted that a 3-L "powerwash" with 2% chlorhexidine effectively decontaminated a bone-tendon cadaveric graft[15]. This discrepancy in effectiveness compared with our data may be explained by the mechanical effect of the powerwash or the difference in tissue types tested.

Regardless of the chosen chemical treatment agent, a mechanical scrub universally prevented positive bacterial culture. Normal saline lavage was not nearly as effective as mechanical scrub for decontaminating fragments and therefore cannot be recommended as the only treatment. Our results augment several previous studies in confirming that an aggressive mechanical scrub can effectively decontaminate bone[15,19,24]. Further study will be required to determine if mechanical brush scrubbing can be employed as the only means of decontamination in the operating room. Consideration will also need to be given to the type of tissue being treated, given the apparent effect of mechanical scrubbing on articular cartilage used in Phase III.

In Phase III, all decontamination procedures were found to decrease cell viability compared with controls. This is critical for the surgeon who requires guidance in determining an appropriate decontamination solution, considering the low regenerative potential of articular chondrocytes. On the basis of our data, we do not recommend 4% chlorhexidine gluconate as an effective decontamination solution for dropped osteoarticular fragments. Also of concern is the finding that a one-minute mechanical scrub resulted in significantly more cell death than normal saline solution lavage. Thus, while a one-minute mechanical scrub effectively decontaminates osteoarticular fragments, it seems to also unnecessarily sacrifice host cell viability. We still do not know to what level this (or any of the other agents) affects osteocyte, in contract to chondrocyte, retention.

The present investigation had several limitations. In Phase I, we did not culture bone fragments that were not dropped on the floor (a negative control). It is possible that a small percentage of presumed "sterile" specimens could produce positive cultures through inadvertent contamination of either the culture media or osteoarticular fragment. This potentially biases our results to overestimate the percentage of positive cultures produced by such fragments contacting the operating room floor. To minimize this effect, our specimens were efficiently harvested in a sterile, in-progress, operative setting. Additionally, in Phase I, culture media were not designed to specifically identify mycobacterial, fungal, or other unusual pathogens. However, given their rarity as infectious pathogens in the United States, our limited resources and personnel necessitated their exclusion.

It is important to note that the clinical importance of a positive tissue culture remains unknown. Several studies have suggested that grafts with intraoperative positive cultures do not necessarily progress to clinical infection[6,25-27]. However, those studies did not examine situations with a known contamination event, which may increase the likelihood of a clinically important infection. Although the present study did not address the long-term infectious risk posed by replanting contaminated bone into humans, we believe culture-positive growth from the operative environment can be considered to be a reasonable marker for risk of future surgical site infection.

While Phase I attempted to replicate an actual contamination event that may be encountered, Phase II relied on controlled laboratory inoculations. The latter method was chosen to maximize the expected yield of contamination. Because of the rarity of gram-negative rod colony growth in Phase I and limited resources, this potential contaminant was excluded from evaluation in Phases II and III. In Phase III, only chondrocyte viability was evaluated. We did not specifically assess the viability of osteocytes, osteoblasts, or osteoclasts. However, as cartilage represents the only truly irreplaceable cell type of the osteoarticular fragment, we believed that determining chondrocyte viability was most important. Despite histologic evaluation of cell viability, we cannot be sure of the in vivo effect of decontamination on the long-term performance of articular cartilage. Finally, the fragments for the present study were obtained during primary knee arthroplasties. Although we consider it advantageous and appropriate to have tested osteoarticular specimens from in vivo harvested tissue in a true operative setting, the viability and the physiology of the fragments may differ from those produced or dropped during other operative settings. When extrapolating these findings to other institutions, consideration of varying bacterial spectra as well as differences in virulence and resistance is recommended.

When a surgeon is faced with the predicament of a contaminated, indispensible osteoarticular graft, our data suggest that some methods may be better than others for ensuring decontaminated reimplantation, and that there is a trade-off between decontamination and articular cartilage cell viability. As the morbidity associated with any orthopaedic infection can be devastating, we continue to recommend decontamination as a priority above cell viability, although our goal in performing the present study was to enable the optimization of both. Our data suggest that a five-minute povidone-iodine (Betadine) bath, followed by a one-minute bulb irrigation lavage with normal saline solution ("rinse"), is sufficient to decontaminate a dropped osteoarticular fragment, without undue autologous cartilage cell toxicity prior to reimplantation. We do not recommend using 70% isopropyl alcohol/2% chlorhexidine gluconate to decontaminate osteoarticular fragments as it failed to fully sterilize these autografts, even with a ten-minute exposure. Furthermore, we do not recommend either 4% chlorhexidine or a mechanical scrub as they significantly decrease chondrocyte viability. ■

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