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ORIGINAL ARTICLE

What if an articular bone fragment drops on the floor in the course of osteosynthesis? An experimental study

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ABSTRACT

Objectives: This study aimed to evaluate the effectiveness of a 1-min 10% povidone-iodine immersion in the decontamination of dropped osteochondral fragments.

Materials and methods: Forty-eight sets of sterile osteochondral bone fragments, each consisting of three samples, were prepared from removed femoral heads that would otherwise be discarded during different hip replacement surgeries. Immediately afterward, each set was dropped on the floor right behind the surgeon in another operating room in which fracture fixation operations were being performed. Samples were picked up with sterile gloves. A swab culture of the floor was taken. One of the three pieces was kept as the control group. The second one (saline group) was washed with saline and subsequently soaked in saline for 1 min. The last one (treatment group) was first immersed in a 10% povidone-iodine solution for 1 min, then rinsed with saline and soaked in saline for 1 min. The samples were cultured in nutrient media, and microorganisms were identified at the microbiology laboratory. The groups were compared in terms of positive culture rates.

Results: The positive culture (contamination) rates were 100%, 58.3%, 39.6%, and 10.4% for the swab samples, control group, saline group, and treatment group respectively. The decontamination ratio in the treatment group was significantly more than both the control group ($p<0.001$) and the saline group ($p=0.001$). Handling only with saline did not significantly decontaminate compared to the control group ($p=0.066$).

Conclusion: Immersing the dropped osteochondral fragments in 10% povidone-iodine solution for 1 min and then rinsing with saline may provide statistically significant decontamination but cannot be accepted to be safe enough for clinical practice. Further studies are needed to find the optimal time needed for safe decontamination without compromising the viability of cartilage tissue.

Keywords: Contamination, decontamination, medical errors, osteoarticular, povidone iodine, surgical attire.

No matter how cautious, unfortunate accidents can happen during surgery, and a bone fragment may inadvertently fall out of the sterile field. This may occur in the course of an osteoarticular autograft transfer procedure or during osteosynthesis, for instance, during open reduction and internal fixation of a radial head fracture. Although the estimated frequency of such an event is extremely rare, at least one in three orthopedic surgeons is likely to encounter this condition during their career.[1]

If the contaminated fragment contains an osteoarticular piece that cannot be discarded or replaced, the surgeon is confronted with a difficult and prompt decision of how to effectively decontaminate and use this piece while preserving the cellular viability of bone and cartilage tissues during decontamination. Saving and reimplanting grafts after proper decontamination is highly recommended in the literature, even for isolated bones.[2]

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The literature includes a number of studies about the fall of various autologous and allograft tissues on the floor, their contact with other nonsterile surfaces, and variable decontamination procedures. However, in most experimental models, to ensure uniformity of contamination among study cohorts, intentional contamination solutions for bacteria were used.[3,4] This study attempted to simulate the situation in a more realistic model to provide guidance on how best to proceed and to evaluate the efficiency of a 1-min 10% povidone-iodine immersion in the decontamination of dropped osteochondral fragments. Secondarily, we aimed to document the incidence of positive culture results in osteochondral fragments that dropped on the operating room floor.

MATERIALS AND METHODS

This prospective experimental study was conducted in the operating room suite of one of the well-established research and training hospitals that is currently closed and out of service. The hospital was a high-volume tertiary trauma center, where six operating rooms were reserved for the orthopedics and traumatology departments. The routine cleaning of environmental surfaces was being performed according to hospital policy, and the floors were being cleaned with a wet mop soaked in disinfectant and water each time before the next patient was taken to the surgical theatre. The data were collected from these rooms throughout 48 different operations on different days between September 2017 and December 2017.

In the study, 48 sets of osteochondral bone samples were prepared. Each set was obtained from a different femoral head removed at the time of primary hip replacement that would have routinely been discarded. Osteochondral fragment specimens from patients with a history of infection, tumor, immunodeficiency, and those undergoing revision hip arthroplasty were excluded. A prophylactic dose of 1 g cefazolin was routinely administered intravenously to all patients 30 min before the induction of anesthesia.

Preparation and contamination of pieces

One of the scrubbed assistants cut up three 1 cm^3 osteochondral pieces (a total of 144 pieces) with a saw using the aseptic technique. These pieces were put into a sterile urine specimen collection cap and passed to the designated person, who proceeded with the contamination and decontamination procedures. The pieces were taken straightaway to one of the other available operating rooms in which fracture fixation operations were being performed. They were dropped on the floor adjacent to the surgical field from a height of 30 cm to keep them close to each other and picked up in 15 sec by hand with the sterile gloves on (Figure 1). Each piece was put into a different sterile cap. Additionally, swab cultures of the floor were taken from an approximately 25 cm^2 area around the point where the bone was picked up. The duration of the operation ongoing in that room until that time was recorded.

Decontamination

One of the osteoarticular pieces was put in another sterile cap and kept as the control group. The second one was washed with 1 L of sterile saline and subsequently soaked in saline for 1 min. Then, it was retrieved and put in a different cap. The last one was first immersed in a 10% povidone-iodine solution for 1 min, then rinsed with 1 L of sterile saline and soaked in saline for 1 min (Figures 2, 3). This one was also retrieved and put in another sterile cap. The pieces were handled with sterile gloves each time, and the gloves were changed at each step.

Microbiological assessment

The specimens were swiftly brought to the microbiology laboratory. They were incubated in

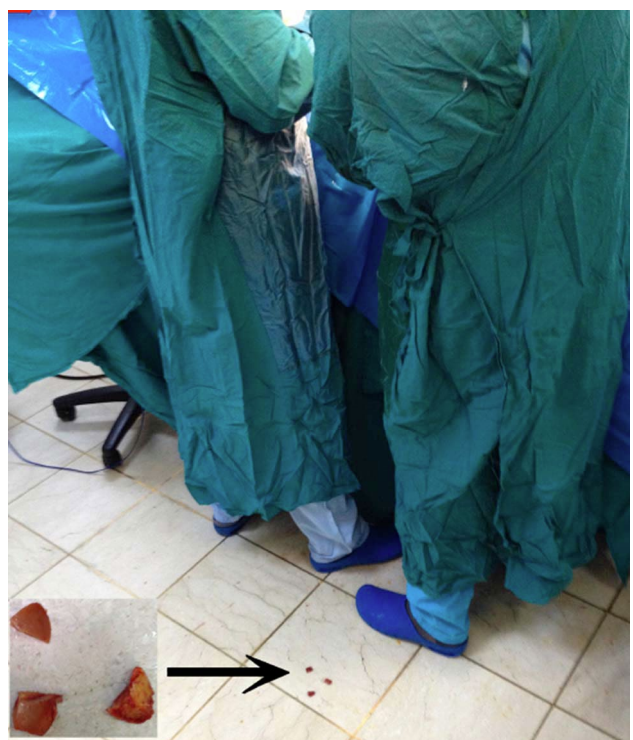

FIGURE 1. Osteochondral bone pieces dropped on the floor just behind the surgeon.

FIGURE 2. Samples immersed in a 10% povidone-iodine solution and in sterile saline.

a brain-heart infusion broth at 37°C. They were manually shaken every 5 min for 30 min in the beginning. After 24 h, the culture solutions were inoculated onto blood agar and eosin-methylene blue agar for another 24 to 48 h. Then appearing colonies were qualitatively evaluated with different staining methods, including gram staining. All bacteria were identified by MALDI-TOF MS (matrix-assisted laser desorption ionization time of flight mass spectrometry (Bruker GmbH, Mannheim, Germany).

Statistical analysis

Statistical analysis was performed using PASW version 18.0 (IBM Corp., Armonk, NY, USA). The data were arranged in 2×2 tables. The operation time was categorized into the 1st or 2nd h. Bacteria were also categorized as nonpathogenic (e.g., coagulase-negative *Staphylococcus*, and *Corynebacterium*) or pathogenic (e.g., *Escherichia coli, Enterococcus, Pseudomonas, and Acinetobacter*). The null hypothesis

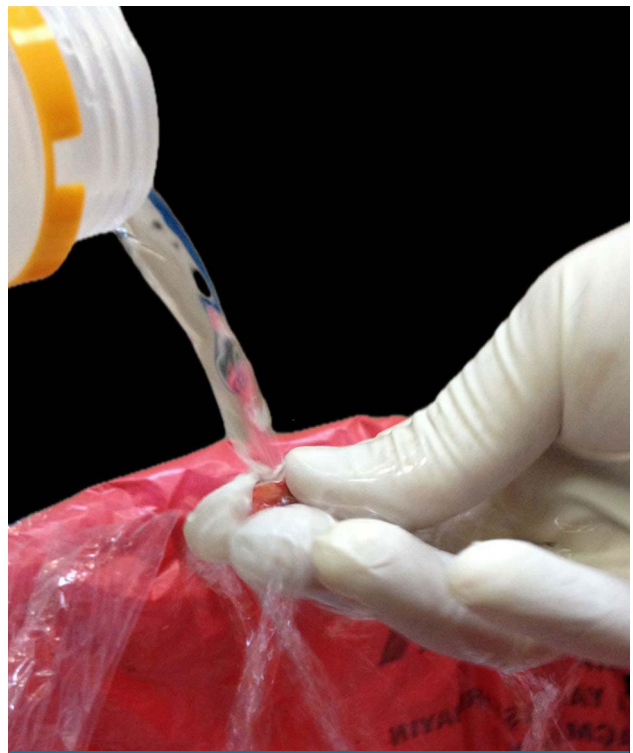

FIGURE 3. Rinsing with 1 L of saline.

that the proportion of sterile fragments following treatment with povidone-iodine + saline was not greater than the proportion of fragments following treatment with saline or the control group was tested. Categorical variables were described as frequencies and compared with the chi-square test. The level of significance was set at $p<0.05$.

TABLE I
Positive and negative culture results of the groups

	Positive		Negative	
	n	%	n	%
Swab samples (n=48)	48	100	0	0
Control group (n=48)	28	58.3	20	41.7
Saline group (n=48)	19	39.6	29	60.4
Treatment group (n=48)	5	10.4	43	89.6

TABLE II
Comparison of positive culture rates

	Saline group	Treatment group
Control group	0.066*	**<0.001***
Saline group	-	**0.001***

* Chi-square test; p<0.05 is in bold.

TABLE III
Microbiological results of the samples

No	Room	hour	Swab culture	Control group	Saline group	Treatment group
1	E	1	*S. epidermidis*	-	-	-
2	D	1	*C. tuberculosterium*	*C. tuberculosterium*	-	-
3	B	2	*S. cohnii + Staf. pasteuri*	*S. cohnii + S. pasteuri*	-	-
4	A	1	*E. coli*	*E. coli*	-	-
5	B	1	*S. epidermidis*	-	-	-
6	A	1	*S. epidermidis*	-	*S. epidermidis*	-
7	B	1	*E. coli + E. faecalis*	-	*E. faecalis*	-
8	F	1	*S. haemolyticus*	*S. hominis*	-	-
9	E	1	*S. epidermidis + S. hominis*	*S. hominis*	-	-
10	A	2	*S. haemolyticus + S. hominis*	-	-	-
11	C	1	*S. epidermidis*	-	-	-
12	A	1	*S. hominis*	*S. epidermidis + S. haemolyticus*	-	-
13	D	2	*S. hominis + Proteus spp.*	*S. hominis*	-	-
14	E	1	*S. epidermidis*	-	-	-
15	D	2	*S. epidermidis + S. hominis*	*S. epidermidis*	*S. hominis*	-
16	E	1	*S. lugdunensis*	-	-	-
17	D	2	*S. epidermidis*	-	-	-
18	B	2	*S. simulans*	-	*S. epidermidis*	-
19	B	2	*S. hominis + S. capitis*	*E. faecalis*	*E.faecalis*	*S. warnerii*
20	C	1	*S. epidermidis*	*S. epidermidis*	*S. epidermidis*	*S. epidermidis + S. haemolyticus*
21	A	2	*E. feacalis + S. capitis*	*E. faecalis*	-	-
22	E	1	*E. feacalis + S. capitis*	*S. epidermidis + S. salivarus*	*E. faecalis + S. capitis*	-
23	D	1	*S. saprophticus + S. epidermidis*	*S. saprophticus + S. epidermidis*	*E. faecalis*	*S. hominis*
24	A	2	*S. epidermidis + S. haemolyticus*	*S. hominis*	*S. capitis*	*S. epidermidis + S. haemolyticus*
25	C	1	*S. capitis*	-	-	-
26	C	1	*S. epidermidis*	*S. epidermidis*	-	-
27	A	1	*S. epidermidis + S. hominis*	-	*B. lincheniformis*	-
28	D	2	*S. pausteri*	*S. mitis*	-	-
29	C	2	*S. hominis*	*S. haemolyticus*	*S. haemolyticus*	-
30	C	2	*S. epidermidis + S. haemolyticus*	*S. epidermidis*	-	-
31	B	2	*S. hominis*	-	-	-
32	D	1	*S. epidermidis*	-	-	-
33	D	1	*S. epidermidis*	*E. faecalis*	*E. faecalis*	*E. feacalis + C. tropicalis*
34	A	2	*S. hominis*	*E. faecalis*	*E. faecalis*	-
35	F	1	*S. epidermidis*	*C. afermentas*	*C. afermentas*	-
36	E	2	*S. epidermidis*	*S. epidermidis*	-	-
37	D	1	*S. epidermidis*	-	-	-
38	C	1	*S. hominis*	-	-	-
39	A	2	*S. epidermidis + S.hominis*	*S. epidermidis*	*S. hominis*	-
40	B	1	*S. epidermidis*	*S. epidermidis*	-	-
41	D	1	*S. epidermidis*	-	*S. simulans*	-
42	B	1	*S. epidermidis*	*S. hominis*	*S. lugdinensis*	-
43	A	1	*S. epidermidis*	*S. hominis*	*S. haemolyticus*	-
44	B	1	*S. epidermidis*	-	-	-
45	C	2	*S. hemolyticus*	-	-	-
46	E	2	*S. haemolyticus + S. epidermidis*	*S. haemolyticus*	*S. haemolyticus*	-
47	D	1	*S. epidermidis*	*S. epidermidis*	-	-
48	E	1	*S. epidermidis*	-	-	-

RESULTS

The positive culture rates (contamination rates) were 100%, 58.3%, 39.6%, and 12.5% for the swab samples, control group, saline group, and povidone-iodine+saline group (treatment group), respectively (Table I). The povidone-iodine significantly decontaminated the osteochondral fragments compared to both the control group ($p<0.001$) and the saline group ($p=0.003$). Isolated saline did not significantly decontaminate the osteochondral fragment compared to the control group ($p=0.066$, Table II).

The microbiological results of whole samples are given in Table III. The most frequent microorganism identified among the swab samples was *Staphylococcus epidermidis* (58.3%). The identified pathogenic organisms were *E. coli* (4.2%) and *Enterococcus faecalis* (6.3%). The other microorganisms were *Corynebacterium tuberculosterium*, *Staphylococcus cohnii*, *Staphylococcus pasteuri (S. pasteuri)*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus lugdunensis*, *Staphylococcus simulans*, *Staphylococcus capitis*, and *Staphylococcus saprophycitus*. More than one species grew on 33.3% of all swab samples. Pathogenic microorganism contamination rate was 8.3%.

The most frequent microorganism identified in the control group was *S. epidermidis* (20.8%). The identified pathogenic organisms were *E. coli* (2.1%) and *E. faecalis* (8.3%). The other microorganisms were *C. tuberculosterium*, *S. cohnii*, *S. pasteuri*, *S. hominis*, *S. haemolyticus*, *Streptococcus salivarius*, *S. saprophycitus*, *Streptococcus mitis*, and *Corynebacterium afermentas*. Pathogenic microorganism contamination rate was 10.4%. The control group specimens exhibited different bacterial profiles in 81.3% of the samples compared to the swab culture that was taken from the same spot.

The most frequent microorganism identified in the saline group was *E. faecalis* (12.5%). No other pathogenic organisms were detected in the group. The remaining microorganisms identified were *S. epidermidis*, *S. hominis*, *S. capitis*, *Bacillus lincheniformis*, *S. haemolyticus*, *C. afermentas*, *S. simulans*, *and S. lugdunensis*.

The most frequent microorganism identified in the povidone-iodine+saline group was *S. haemolyticus* (4.2%). Pathogenic microorganisms *(E. faecalis and Candida tropichalis)* were detected in one sample (2.1%). The remaining microorganisms identified in the cultures were *S. epidermidis*, *Staphylococcus warnerii*, *S. haemolyticus*, *and S. hominis*.

In all groups, the vancomycin resistance test results for *E. faecalis* microorganisms were negative. Samples dropped 2 h after the start of operations had higher contamination rates compared to those dropped after 1 h. However, the difference did not reach statistical significance (44.4% *vs.* 32.2%, $p=0.141$). There was no statistically significant association between culture positivity and the room where the culture was collected ($p=0.678$).

DISCUSSION

Accidental dropping of an irreplaceable osteochondral bone fragment on the floor poses a challenging dilemma to the surgeon, and there are no accepted guidelines for the management of this undesirable situation. In the present study, we aimed to create the most realistic scenario to find if a 1-min 10% povidone-iodine immersion of dropped osteochondral fragment is sufficient to overcome this difficulty. We found that washing and immersing the dropped osteochondral fragments only with saline did not efficiently decontaminate the dropped pieces, but immersing them in a 10% povidone-iodine solution for 1 min provided decontamination in 10.4% of the incidents. Povidone-iodine is an antiseptic with bactericidal, virucidal, fungicidal, and mycobactericidal properties.[5]

There is high variability in the reported contamination rates of dropped bones. In the study of Presnal and Kimbrough,[6] the authors surprisingly reported a 0% contamination rate among bone grafts dropped on the operating room floor and concluded that dropped bone grafts may be used without decontamination. Bruce et al.[3] reported a 70% contamination rate for dropped osteochondral fragments. Mat-Salleh et al.[7] reported that the risk of contamination of a dropped bone during surgery is 86.5%. Alomar et al.[2] reported a 42% contamination rate in dropped osteochondral autografts. In our study, the control group had a %58.3 contamination rate. *Staphylococcus epidermidis* was the most frequently cultured microorganism in the current study, consistent with the literature.[2,3]

Culture positivity rate of the pathogenic microorganisms was relatively low in our swab samples compared to the cumulative positive culture rate (8.3% *vs.* 100%). Similarly, in the control group, these rates were 10.4% *vs.* 58.3%. As pathogenic ones could have a small colony count and be outgrown by nonpathogenic microorganisms due to competition for nutrition and various growth necessities in the culture media, the real contamination rates of pathogens, particularly in these two groups, might

TABLE IV
Comparison of our series with recent literature regarding povidone-iodine treatment for the decontamination of contaminated bones

	n	Decontamination duration	Washing with saline	Contamination method	Positive culture rate	Histopathological analysis of cells	Studied specimen
Current study (2024)	48	1 min	Yes	Dropped fragment	10.4%	No assessment	Osteochondral fragment
Altınayak et al.[14]	30	15 min	No	Dropped fragment	0%	No assessment of cartilage tissue	Osteochondral fragment
Mat-Salleh et al.[7]	45	10 min	Yes	Dropped fragment	67.6%	No assessment	Isolated bone
Yazdi et al.[11]	50	20 min	Yes	Dropped fragment	0%	No assessment	Rabbit bone
Bruce et al.[3]	28	5 min/10 min	Yes	Inoculating with Coagulase negative staphylococcus	0%	Yes (51% live chondrocytes)	Osteochondral fragment
Bauer et al.[4]	10	2 min	Yes	Bacterial broth	60%	Yes (66% live bone cells)	Isolated bone

be underestimated. This may be the reason why no difference was observed between saline and treatment groups in terms of pathogen microorganism contamination. Furthermore, 81.3% of the control group exhibited different culture results compared to swab samples taken from the same spot. In light of these findings, we believe that microbiological evaluations of orthopedic operation rooms by swab cultures do not necessarily identify microorganisms that can grow in cultures of fallen bone samples.

In the literature, there are several studies that used intentionally contaminated bacterial solutions. Stanford et al.[8] used bacterial solutions that were intentionally inoculated with *Staphylococcus aureus* and *Pseudomonas aeruginosa* to contaminate bone-tendon grafts that were obtained from cadavers. The authors concluded that 30 min of immersion with povidone-iodine is not sufficient for decontamination. The reason for this insufficiency may be associated with the high bacterial load of the solution as well as the use of highly resistant microorganisms. Another study showed that when the number of microorganisms inoculated decreases, the antiseptic exposure time needed also decreases.[9] Additionally, Alomar et al.[2] showed that the contamination levels (CFUs/g) were very low in the dropped bone fragments. Considering the viability of cartilage tissue is crucial for osteosynthesis surgeries, and the real contamination level is expected to be lower than intentionally inoculated bacteria solutions. Thirty minutes of povidone-iodine treatment might be much longer than what is needed. Bruce et al.[3] found no difference between 5 min and 10 min of exposures to povidone-iodine, and they stated that shorter exposures may also provide sufficient decontamination. It has been shown that only 2 min of exposure to povidone-iodine or chlorhexidine can cause almost immediate cell destruction.[10] To our knowledge, we used the shortest povidone-iodine exposure time (1 min) compared to the literature. One minute of povidone-iodine treatment, followed by 1 L of saline rinsing and then 1 min of saline immersion, provided significant decontamination in our study. Table IV shows a comparison between our study and the recent literature regarding povidone-iodine treatment.

Bruce et al.[3] used osteochondral pieces that were obtained from total knee arthroplasty. The authors concluded that 5 min of immersion in povidone-iodine and 1 min of rinsing with saline provided the optimum decontamination-cellular viability balance. Bauer et al.[4] also used

bones obtained from total knee arthroplasty in their study. They washed the bone pieces with povidone-iodine for 2 min, then waited for 15 min to dry the povidone-iodine and rinsed with saline. They stated that this procedure provided optimum decontamination-cellular viability balance. On the other hand, the study reported that only 21% of the cells were alive with this procedure. However, both of these two studies were performed using intentionally inoculated solutions for contamination.

Later studies tried to create a more realistic experimental model and dropped the grafts or bones in the operating rooms to better simulate the situation. Yazdi et al.[11] used rabbit osteochondral bones. They dropped the bone pieces in the operating rooms and waited for 15 sec. They reported effective sterilization with 20-min povidone-iodine, antibiotic, and chlorhexidine solutions. The main problem with these treatment methods appears to be the possibility of destroying the structures of cells and tissues.

Barbier et al.[12] dropped hamstring tendon grafts in the operation room, and they suggested 15 min of povidone-iodine or chlorhexidine treatment. Moreover, a recent review, including anterior cruciate ligament grafts, concluded that 4% chlorhexidine efficiently disinfected the grafts.[13] Nevertheless, the contamination level of hamstring grafts is different from that of osteochondral fragments, and these tendon grafts do not include cartilage or bone cells that definitely should be protected from cellular toxicity.

Altınayak et al.[14] dropped bones in the operating room after the knee arthroplasty procedure was completed. They reported efficient decontamination by immersing the bone in the povidone-iodine solution for 15 min without rinsing with saline. Despite reporting no decrease in osteoblastic activity, they did not perform the histopathological examination of cartilage tissue, which represents the only truly irreplaceable cell type of the osteochondral fragment. As Bruce et al.[3] previously stated, long exposure durations with povidone-iodine may cause cellular toxicity in the chondrocyte cells. We used a 1-min exposure duration, subsequently rinsed with saline, and then soaked the fragment in a saline solution to minimize the toxic effects of povidone-iodine on both cartilage and bone cells.

Mat-Salleh et al.[7] used bones obtained from knee and hip arthroplasties and washed the bones for 10 min with povidone-iodine or chlorhexidine and recommended the chlorhexidine treatment, but they did not evaluate cellular toxicity. Furthermore, Özbay et al.[15] found that a 0.05% chlorhexidine solution used as an irrigative did not affect fracture healing in a rat femur fracture model. On the other hand, there are many studies in the literature reporting the cytotoxicity of chlorhexidine solutions.[3,4,16] In the study of Bruce et al.,[3] 1-min exposure of bone fragments to chlorhexidine caused nearly total chondrocyte death.

The guidelines and recommendations for preventing surgical site infections, such as reducing foot traffic in the operating room, are well known, but it is a fact that these rules are followed more strictly in arthroplasty operations.[17,18] Therefore, we dropped the bones in the operating rooms where trauma surgeries were ongoing as there was heavy foot traffic due to radiology staff and observers. This made our study more realistic. Operating room staff have been shown to be a source of floor flora in the operating rooms.[19] The quantity of bacteria is directly related to the number of people present in the room.[20] In the present study, although it is strictly prohibited by hospital regulations, operating theatre clogs that were worn outside the sterile field (e.g., toilets) may be a possible reason for the presence of *E. faecalis* and *E. coli*.

We chose a realistic contact time (15 sec) with the floor and aimed to mimic an actual osteochondral fragment contamination event. Additionally, dropping the fragments on various operation room floors at various times instead of dropping at a single operation room and at a single time point can be considered another strength of the study.[21] Mat-Salleh et al.[7] demonstrated that the bacterial load on the operating room floor amplifies as the time of surgery progresses. In our study, the higher operative duration group exhibited nonsignificantly higher rates of positive cultures.

Moreover, we used osteochondral tissue instead of an isolated bone, as this would be more similar to the scenario in which an indispensable osteochondral piece was dropped, such as a radius head or talus fragment in a trauma case.

This study has several limitations. First, decontamination with only 10% povidone-iodine solution and only 1-min immersion is examined. Second, the viability of the cells was not checked after the procedure. We used the shortest exposure duration in the literature and also rinsed and soaked with saline immediately after immersing with povidone-iodine in the light of literature knowledge.[3,4] We believe that our procedure may

not cause significant cellular toxicity. Applying a higher duration of povidone-iodine, or mechanical scrubbing may cause cellular toxicity in bone and cartilage tissues.[3,4,10] Third, quantitative analysis of microorganisms in cultures was not performed. Finally, the present study does not directly address the clinical infection risk after decontamination procedures. Osteochondral fragments with positive cultures do not necessarily progress to clinical infection. However, these positive culture results can be considered a reasonable predictive factor for increased future surgical site infection.

In conclusion, if an articular bone fragment drops on the floor in the course of osteosynthesis, immersing the dropped osteochondral fragments in 10% povidone-iodine solution for 1 min and then rinsing with saline would save this indispensable fragment in most cases, but the clinical results of this protocol need to be further investigated. Nevertheless, it should not be forgotten that full compliance with operating room rules is a must at all times.

Ethics Committee Approval: The study protocol was approved by the Ankara Numune Training and Research Hospital Scientific Research Evaluation Commission (date: 27.05.2015, no: 2015-982). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept, design, data collection, interpretation, literature review, writing the article: A.U.; Data collection and processing, materials: G.H.; Analysis and interpretation, writing the article : Z.E.Ç.; Materials, literature review: C.C.K.; Supervision, critical review: B.Ö.

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