

## Effect of three different sterilizing solutions on the contaminated bone: an experimental study in the rabbit

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### Abstract

**Background:** To determine the efficacy of three different antiseptic solutions (Control group (I), Antibiotic solution – Neomycin and polymyxin (II), Chlorhexidine 0.4% (III), and povidone – iodine 10% (IV)) in disinfecting contaminated bone fragments.

**Methods:** Under sterile conditions, the femora of 12 rabbits were removed and cut into six millimeter pieces. A total of 200 bone specimens were obtained. All 200 specimens were dropped on the operating room floor for fifteen seconds and assigned to one of four experimental groups.

Group I samples were cultured after immersion in normal saline solution (Control group). In other three groups, prior to culture the samples, they were washed with normal saline for ninety seconds and placed in an antibiotic solution (Neomycin & Polymyxin) (group II), Chlorhexidine 0.4% (group III), and povidone-iodine 10% (group IV) respectively.

**Results:** In group I, 22 of 50 specimens had positive cultures. Of 50 specimens of group II and IV, positive cultures were found in 3 and 2 grafts respectively after 10 days whereas no positive cultures were detected in any samples of group III.

**Conclusion:** Chlorhexidine 0.4% seems to be the best antiseptic solution for decontaminating the contaminated bone samples although it did not have any significant difference with povidone-iodine and other antibiotic solution.

**Keywords:** Sterilizing solutions, Contaminated bone.

### Introduction

The intraoperative contamination of a bone fragment during any orthopaedic surgery such as dropping the specimen on the floor could occasionally occur and create a serious dilemma for the surgeon. If the autograft is contaminated this way on the operating room floor during the surgery, salvage can be done by different methods: 1. A disinfection technique for the contaminated fragment; 2. the use of allograft; 3. harvesting

another graft from other sites in some cases which accompanied with additional risks to the patients (1).

Little data are available concerning the incidence of positive cultures from the dropped bone or the efficacy of methods of disinfecting techniques on the contaminated bones (2,3,4). Most of previous researches were conducted on the knee ligament grafts which they did not consider bone specimens only.

The logical solution for decontamination of

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the specimens is autoclaving and formaline treatment, which reduce the number of organisms significantly. Unfortunately, it has deleterious effect on graft material. In other words, the ideal method of decontamination is applying a disinfectant which destroys the organisms without any harmful effect on the bone and osteogenic cells.

To the best of the authors' knowledge, there is no documented study regarding the management of the contaminated bone fragment in the English orthopaedic literature. The purpose of the recent study was to document the incidence of positive cultures from the bone fragment that have been dropped on an operating room floor and to determine the efficacy of different antiseptic solution in disinfecting contaminated bone fragments.

### Methods

*Animals and surgical techniques:* Twelve rabbits weighing between 2000 and 3000 g (mean, 2500±500) were housed in Shafa Rehabilitation hospital's animal facility. They were maintained on a 12 hour light-dark cycle. Intramuscular anesthesia with Ketamine and Midazolam was performed in the supine position.

The inner sides of the both thighs up to abdomen were thoroughly shaved and prepared with 10% povidone-iodine. After making the skin incision under sterile conditions, the femora were reached through blunt dissection. After removing the femora and complete detaching of the relevant soft tissue, rabbits were sacrificed through intracardiac injection of potassium chloride.

All femora were cleansed of soft tissue thoroughly and cut into six-millimeter pieces. A total of 200 bone specimens were obtained from 12 rabbit femora.

*The site of the experiment:* In order to simulate an intraoperative environment, the study was conducted in an operating room at the Shafa Rehabilitation Hospital immediately after the completion of a 1.5-hour arthroscopic ACL reconstruction.

*Experimental groups:* Pre-contamination cultures of all 200 specimens were performed with no positive cultures detected in this stage.

All 200 specimens were dropped on the operating room floor from the height of operating table, where they were allowed to remain for fifteen seconds, which is the average time to find dropped specimen in our study. Each specimen was then picked up with sterile forceps and alternatively assigned to one of four experimental groups.

After undergoing contamination, all specimens were divided into four groups and immersed in different disinfecting solutions. *In group I*, each specimen was immediately washed with normal saline for 1.5 minutes and immersed into normal saline solution for twenty minutes. They were then placed into individual sterile specimen cups containing culture medium (Thioglycolate broth). Fragments of the *group II* were washed with normal saline for ninety seconds and placed into individual containers of an antibiotic solution. (The solution contained 40 mg/ml of neomycin sulphate and 200000 U/ml  $\beta$  – polymyxin). Again they were rinsed with sterile saline for three minutes and then placed into individual cups containing culture medium Thioglycolate broth). *In group III*, the specimens were rinsed with sterile saline for 1.5 minutes and then placed into individual containers of chlorhexidine (0.4% Chlorhexidine gluconate with 4% isopropyl alcohol in nonalkaline base) for twenty minutes. They were then placed into cups containing the media after washing with normal saline for three minutes.

Apart from the solution applied in the previous group, identical treatment was performed on *group IV*. (The solution used in the latter group was %10 povidone-iodine). The saline solution was prepared separately for each graft to avoid cross – contamination in every group.

In addition to four groups, fifty specimens including the operating room swab was also taken to the institute of microbiology for identifying the positive cultures of the operating room.

Table 1. Culture results.

Groups	No. of cultures	Positive cultures (Number)	Positive cultures (%)
I (Control)	50	22	44%
II (Antibiotic Solution)	50	3	6%
III (Chlorhexidine)	50	0	0%
IV (Povidone – Iodine)	50	2	4%
Floor Swab	50	35	70%

The graft cups were then taken to the department of microbiology of Pasteur institute of Iran in order to be incubated at 37°C for 10 days. They were cultured for different organisms including anaerobic and aerobic organisms.

*Statistical Analysis:* Four groups were compared using Chi Square analysis. Due to low number of positive culture samples, Fisher Exact test was used. Frequency percentages are presented in Table 1.

### Results

The results of the study are summarized in Table 1.

Of the fifty specimens in *group I* (Contamination and rinsing in sterile normal saline), 22 grafts (44%) had positive cultures of Staphylococcus species at 10 days.

In *group II* (Contamination followed by antibiotic solution), positive cultures were found in 3 specimens (6%) after 10 days. One sample had Staphylococcus epidermidis while the other two had Gram positive Bacillus.

Among all groups, samples of *group III* (Chlorhexidine solution) had negative cultures at 10 days.

Of all specimens in *group IV* (Contamination followed by povidine-iodine), 2 (4%) positive cultures were found, with Bacillus and Klebsiella species respectively.

Of fifty operating room swabs, 35 (70%) positive cultures were found. They were contaminated with Staphylococcus epidermidis in 22, Gram negative and positive Bacillus in 10 samples respectively, Enterobacter in 2 specimens and Klebsiella in 1.

The standard chi square analysis revealed no statistical significance among different groups of sterilizing agents (Povidine-iodine,

antibiotic solution and chlorhexidine) ( $p$  value = 0.153; Likelihood ratio = 2.813) however samples rinsed in chlorhexidine had not grown cultures.

### Discussion

During any orthopedic surgery, contamination of tissues may occur if it falls on the operating room floor. Presnal and Kimbrough (5) dropped autogenous graft onto the floor of the surgical ward intentionally at different times. Culturing the specimens was done in order to determine the amount of contamination that occurred. Surprisingly, they revealed no positive cultures and found that grafts dropped onto the floor may have no decontamination. However, Molina et al (1) revealed positive floor swab cultures in 48 (96%) of 50 specimens. In our study, 70% of 50 floor swab cultures were positive.

In order to decontaminate the specimens, we chose the common disinfectants used in surgical wards. In one hand, Hooe and Steinberg (6) showed that Neomycin – polymyxin solution (40 mg neomycin + 200000 U polymyxin) has little effect on bacteria. On the other hand, Molina et al (1) declared that ACL grafts rinsed in these solutions had 3 positive cultures of 50 specimens (6%). Deijkers et al (7) showed that immersing contaminated grafts in the antibiotic solution may have an effect at low pathogenicity, but no effect at high pathogenicity. Our study revealed that the antibiotic solution has an effect in disinfecting the contaminated bones although there is some residue of positive cultures.

Regarding povidone-iodine, Severyns et al (8) showed that even low concentrations of a povidone-iodine 10% has toxic and deleterious effect on granulocytes and monocytes although Soyer et al (9) reported that it has

efficacy for bone graft decontamination. In the recent study, a 10% povidone-iodine was identified as a decontaminating agent for contaminated bone grafts although positive cultures were found in 4% of cases.

Concerning chlorhexidine as a disinfectant agent, Goebel et al (3) reported that 4% chlorhexidine alone has no effect on bacteria whereas Molina (1) stated that this agent is the most effective agent in sterilizing the contaminated samples. We applied 0.4% chlorhexidine in our study due to possible cytotoxic effect of 4% chlorhexidine. Interestingly, in the present study, it was revealed that 0.4% chlorhexidine is the most effective agent in decontaminating bone plugs with no positive culture although it had no superiority on other agents when compared with other studied solutions.

Surprisingly, the group I (The control group) culture positive rate (50%) was different from that of the floor swab group (70%). The reason was not clear but it may be due to specimens washing with normal saline solution.

In contrast to Molina's study<sup>1</sup> and other previous researches (2,3,10,11), we studied the different decontamination methods of bone fragments. To the best of the authors' knowledge, such a study on the contaminated bone specimen were not previously reported in the literature.

Several limitation of this study should be considered. First, no comparison among different exposure times and solution concentration was made in order to determine the least effective time of exposure and the agent concentration. Second, no histological study of the specimens after exposure to the disinfecting solution was performed to detect any structural changes within the sample cells.

More research involving exposure time is required in order to determine the possible negative effects that these solutions could cause to the tissues.

### Conclusion

Our study shows that 0.4% chlorhexidine seems to have the best antiseptic effect for

disinfecting the contaminated bone samples although it does not have any significant difference with povidone-iodine and the antibiotic solution statistically.

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