

## Contaminated osteochondral plugs: effect of different sterilizing solutions: an experimental study in the rabbit

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### Abstract

**Background:** To determine the efficacy of different antiseptic solutions (Control group (I), Antibiotic solution (II), Chlorhexidine 0.4% (III), and povidone – iodine 10% (IV)) in sterilizing contaminated osteochondral plugs.

**Methods:** Under sterile conditions, the femoral head and condyles of 20 rabbits were removed and cut into equal osteochondral pieces. A total of 200 osteochondral 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 washing with normal saline solution (Control group). In other three groups, prior to culturing process, samples were placed in an antibiotic solution after washing with normal saline (Neomycin & Polymyxin) (group II), Chlorhexidine 0.4% (group III), and povidone – iodine 10% (group IV), respectively.

**Results:** In group I, 25 of 50 specimens had positive cultures. Of 50 specimens of group II, III and IV, no positive cultures were found after 10 days.

**Conclusion:** all three agents including antibiotic solution, povidone-iodine 10% and chlorhexidine 0.4% seem effective in sterilizing the contaminated osteochondral samples. According to the literature, povidone-iodine has no negative effect on the cartilage metabolism and seems to be a proper choice of decontaminating solution for osteochondral plugs. To the best of the authors' knowledge, such a study on the contaminated osteochondral specimen has not been previously reported in the literature.

**Keywords:** Sterilizing, Contaminated, Osteochondral

### Introduction

The intraoperative contamination of an osteochondral specimen during any articular reconstruction surgery can occur and requires the surgeon to make a difficult decision. If the sample is contaminated by being dropped on the operating room floor during the surgery, salvage can be done by different methods. If taking a new specimen from the

patient is not possible, then the surgeon should find a technique to decontaminate the specimen.

Several studies have declared irrigation with different solutions may have a significant effect on the metabolism of the cartilage and result in the morphologic defects (1-3).

Little data are available concerning the incidence of positive cultures from the dropped specimen or the efficacy of sterili-

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zation techniques on the contaminated specimen (4,5).

To the best of the authors' knowledge, no documented study regarding the management of the contaminated osteochondral fragment was found in the English orthopedic literature. The purpose of the recent study was to document the incidence of positive contaminated cultures from the osteochondral specimen that was dropped on an operating room floor and to determine the efficacy of different antiseptic solution in sterilizing contaminated fragments.

## Methods

### *Animals and surgical techniques:*

Twenty rabbits weighing between 2000 and 3000 g (mean  $2500 \pm 500$ ) were housed in Shafa Rehabilitation hospital's animal facility. They were maintained on a 12-hour light-dark cycle. Intramuscular anesthesia with Ketamine (20 mg/kg) and was performed in the supine position.

The inner sides of the both thighs up to the abdomen were thoroughly shaved and prepared with 10% povidone-iodine. After making the skin incision under sterile conditions, the knee and hip articular surfaces were reached through blunt dissection. Femoral head and condyles were sawed in equal-sized blocks. During the procedure, the articular surface was rinsed with sterile distilled water. Using the method of Lipshitz and Glimcher<sup>6</sup>, 5-millimeter cylindrical osteochondral plugs were drilled from the head, the medial and lateral condyles of the femur. During the preparation of the plug, only 2.5 mm of subchondral bone was left under the cartilage. After finishing the procedure, rabbits were sacrificed through intracardiac injection of potassium chloride. A total of 200 osteochondral specimens were obtained from 20 rabbits.

### *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 90-minute arthroscopic (Anterior Cruciate

Lig) ACL reconstruction.

### *Decontaminating solutions:*

Four different sterilizing agents with four groups (I – IV) were considered for the immersion of osteochondral blocks. They are summarized in Table 1.

### *Experimental groups:*

Pre-contamination samples of all 200 specimens were cultured. No positive culture was detected in this stage.

All 200 specimens were dropped on the operating room floor from the height of operating table separately and allowed to remain on floor for fifteen seconds. The average time for each dropped specimen was found in our study. Each fragment was then picked up with sterile forceps and alternatively assigned to each of four experimental groups.

After induction of contamination, all specimens were divided into four groups and immersed in different solutions. In group I, each specimen was immediately washed with normal saline solution for 1.5 minutes and then immersed into normal saline for twenty minutes. Specimens were then placed into individual sterile cups containing culture medium (Thioglycolate broth). Specimens of the group II were placed into individual containers of aneomycin-polymyxin B solution (Neomycin Sulphate 40 mg/ml +  $\beta$ -polymyxin 200000 U/ml) after 1.5-minute washing with normal saline. Again they were rinsed with sterile saline for 1.5 minutes and then placed into individual cups containing culture medium (Thioglycolate broth). In group III, specimens were rinsed with sterile saline for 1.5 minutes and then placed into individual containers of chlorohexidine 0.4% for twenty minutes. They were placed into cups containing the medium culture after washing with normal saline for 1.5 minutes.

Apart from the solution applied in the previous group, the same procedure was performed for group IV (The solution used in the latter group was povidone-iodine 10%). The saline rinse was prepared separately for

Table 1. Composition of sterilizing solutions applied in the study.

Solution	Composition
Group I (Control group)	Normal Saline
Group II	Neomycin Sulphate 40 mg/ml + $\beta$ -polymyxin 200000 U/ml
Group III	Chlorhexidinegluconate 0.4% with isopropyl alcohol 4% in nonalkaline base
Group IV	Povidone – iodine 10%

each graft to avoid cross – contamination in every group.

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

The culture cups were then taken to 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 Fisher exact test since the number of positive cultures were zero (less than 5) in some groups. A p-value less than 0.05 was considered to be statistically significant difference between groups.

#### **Results**

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

Of fifty specimens in group I (Contamination and rinsing in sterile normal saline), 25 grafts (50%) had positive cultures at 10 days. All 25 specimens were *Staphylococcus* species.

All samples of other groups (II, III, IV) had negative cultures at 10 days.

The standard Chi square analysis revealed no statistical significance different among various groups of sterilizing agents (povidone-iodine, antibiotic solution and chlorhexidine) (p value = 0.315). Since all samples rinsed in solutions of group II, III,

IV study are summarized in Table 2.

#### **Discussion**

During any orthopedic surgery, contamination of the specimen may occur when it falls on the operating room floor. Presnal and Kimbrough 7 dropped autogenous graft on the floor of the surgical ward intentionally at different floor-remaining times. Bacterial culturing of dropped specimens were done in order to determine the quality and quantity of contamination that occurred. Surprisingly, they revealed no positive cultures and found that grafts dropped onto the floor may be applied without decontamination. However, Molina et al.8 revealed positive floor swab cultures in 48 (96%) of 50 specimens. In our study, 70% of 50 floor swab cultures were positive.

The logical solution for decontamination of the specimens is autoclave and formaline which reduce the number of organisms significantly. Unfortunately, it has deleterious effect on tissue material. Meanwhile, the ideal method of decontamination is applying a disinfectant which could destroy the organisms without any harmful effect on the tissue.

In order to decontaminate the specimens, we chose the common disinfectants used in surgical wards. In one hand, Hooe and Steinberg9 showed that Neomycin – polymyxin solution (40 mg neomycin + 200000U polymyxin) had little effect on bacteria. On the other hand, Molina et al (8) declared that ACL grafts rinsed in the same had negative cultures. The results of the solution had 3 positive cultures with 50 specimens (6%). Deijkers et al (10) showed that immersing contaminated grafts in the that antibiotic solution may have an effect at level of bacterial number, but no effect at high level. Our study revealed that the mentioned

Table 2. The number of positive and negative culture results.

Groups	No. of cultures	Positive cultures (Number)	Positive cultures (%)
I (Control)	50	25	50%
II (Antibiotic Solution)	50	0	0%
III (Chlorhexidine)	50	0	0%
IV (Povidone – Iodine)	50	0	0%
Floor Swab	50	35	70%

antibiotic solution has an effect in sterilizing the contaminated osteochondral specimen since no positive culture was detected. The question is whether antimicrobial solutions have negative effects on the cartilage vitality. An inhibitory effect on the metabolism of the cartilage may result of prolonged exposure or of excessively high concentration (11).

Regarding povidone-iodine, Severyns et al. 12 showed that even low concentrations of a povidone-iodine 10% has toxic and deleterious effect on granulocytes and monocytes although Soyer et al (13). Reported that it has efficacy of bone graft decontamination. Müller and Kramer showed that iodophores had no deleterious effect on cartilage metabolism. 11 In the recent study, a povidone-iodine 10% was identified as a sterilizing agent for contaminated osteochondral fragment.

Concerning chlorhexidine as a disinfectant agent, Goebel et al. 5 reported that the chlorhexidine 4% solution alone has no effect on bacteria whereas Molina 8 stated that this agent was the most effective agent in sterilizing the contaminated ACL graft samples. We applied chlorhexidine 0.4% in this study because of probability of the cytotoxic effect of chlorhexidine 4%. Interestingly, in the present study, it was revealed that chlorhexidine 0.4% was an effective method in decontaminating osteochondral plugs with no positive culture although it had no superiority when compared with other solutions.

In contrast to previous studies (4,5,8,14) we studied the different sterilization methods for osteochondral fragments. To the best of the authors' knowledge, such a study on the contaminated osteochondral specimen has not been previously reported in the literature.

However, several limitation of this study

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

### Conclusion

Our study shows that all three agents including antibiotic solution, povidone-iodine 10% and chlorhexidine 0.4% were effective in sterilizing the contaminated osteochondral samples. According to the literature, povidone-iodine 10% has no negative effect on the cartilage metabolism and seems to be the choice of decontaminating solution for osteochondral plugs. Further research is required to determine the exposure time and concentration of a sterilizing agent with no negative effect on cartilage metabolism.

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