Comparison of the Sterilization Efficiency of 3 Disinfectants for Dropped Anterior Cruciate Ligament Grafts

A Systematic Review and Meta-analysis

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Background: The inadvertent contamination of anterior cruciate ligament (ACL) grafts can occur if they are accidentally dropped on the floor during ACL reconstruction. There has been no meta-analysis conducted to compare the sterilization efficiency of the different disinfectants used on dropped ACL grafts.

Purpose: To compare the sterilization efficiency of 3 disinfectants to decontaminate ACL grafts as necessary.

Study Design: Systematic review.

Methods: A systematic literature review was performed using the MEDLINE, Embase, and Cochrane Library databases. All studies reporting the management of dropped or contaminated grafts were considered for this meta-analysis.

Results: A total of 7 studies meeting inclusion criteria were identified from a literature search. The pooled results of this meta-analysis indicated that the rate of positive cultures of ACL grafts dropped on the operating room floor was 44.9% and that the commonly contaminated microbes were staphylococci and bacilli. The meta-analysis results indicated that the sterilization efficiency of a 4% chlorhexidine solution was superior to an antibiotic solution (odds ratio [OR], 0.17 [95% CI, 0.05-0.57]; \( P = .004 \)) and a 10% povidone-iodine solution (OR, 0.04 [95% CI, 0.01-0.20]; \( P < .0001 \)). Further, the antibiotic solution was superior to the 10% povidone-iodine solution (OR, 0.20 [95% CI, 0.07-0.55]; \( P = .002 \)).

Conclusion: The results of our meta-analysis demonstrated that staphylococci and bacilli were the most common contaminants on dropped ACL grafts and that decontamination using a 4% chlorhexidine solution more reliably disinfected ACL grafts. This information can help to guide surgeons as regards appropriate remedial measures.

Keywords: sterilization; graft; anterior cruciate ligament (ACL); disinfectant; contamination
decontamination efficiency of different disinfectants on dropped ACL grafts.

This study aimed to provide guidelines for surgeons by comparing the efficiency of 3 disinfectants commonly available in the operating room, a 4% chlorhexidine solution, an antibiotic solution, and a 10% povidone-iodine solution, and identifying the most effective way to disinfect ACL grafts that fell on the operating room floor. We hypothesized that the 4% chlorhexidine solution, antibiotic solution, and 10% povidone-iodine solution would have equivalent efficacy and that using any of them would provide effective sterilization in cases of dropped ACL grafts during surgery.

METHODS

Search Strategy and Eligibility Criteria

This study was registered with PROSPERO, an international database of prospectively registered systematic reviews (CRD42020205369). A systematic review was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. On May 1, 2020, an electronic literature search was performed using the MEDLINE, Embase, and Cochrane Library databases. The search terms used were as follows: anterior cruciate ligament and ACL combined with graft contamination, dropped graft, and sterilization. No restrictions were imposed on the date of publication.

All novel studies evaluating the management of dropped or contaminated grafts were eligible for inclusion in this review. The study exclusion criteria were as follows: non-English language publications, nonexperimental studies, noncomparative studies, and studies unrelated to ACL grafts.

Titles and abstracts were independently reviewed by 2 authors (X.S. and T.L.) to ensure that the selected articles met the inclusion criteria. Any disagreements regarding study inclusion and data were resolved by discussion and consensus involving a senior reviewer (Y.Q.).

Data Extraction

The same 2 reviewers independently extracted all relevant data and imported them into a spreadsheet (Excel 2019; Microsoft), which was then reviewed by another senior reviewer (J.Z.). The imported data included the study's country of origin, study design, sample types, sample size, source of contamination, decontamination management protocol, and outcomes.

Methodological Quality Assessment

The included studies' methodological quality was assessed using the criteria of the modified Methodological Index for Non-Randomized Studies (MINORS). Similar to the original MINORS, the modified MINORS criteria included 12 items, with 2 additional items proposed for future in vitro studies. The total score of the methodological quality assessment was 24. The quality of studies was rated as follows: high quality with 19-24 points, moderate quality with 13-18 points, and low quality with <12 points. All included articles were independently assessed by 2 reviewers (X.S. and J.X.), and any disagreements regarding the quality assessment were resolved by discussion and consensus involving a senior reviewer (J.Z.). The purpose of the quality assessment in this meta-analysis was to evaluate and describe the study quality and design characteristics of all included studies.

Statistical Analysis

Review Manager 5.3 software (Cochrane Collaboration) was used for the meta-analysis of the extracted data. The primary outcome of interest was the odds ratio (OR) of positive cultures. A random-effects model was used to evaluate the random variables and I² statistic to evaluate the data for heterogeneity among studies and confirm the appropriateness of pooling among groups, with >50% regarded as significantly heterogenic. The OR, with a 95% CI, was calculated for dichotomous outcomes. If no events occurred in 2 groups or no comparison between 2 groups could be made within a study, those studies were excluded from this part of the meta-analysis.

RESULTS

Study Characteristics

The literature search yielded a total of 423 articles. After removing duplicate studies and applying exclusion criteria, 30 studies were selected for a full-text review. Based on eligibility criteria, 23 of these studies were excluded after a full-text review, and the remaining 7 articles were ultimately included. A flowchart of the study selection process is shown in Figure 1.

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Quality Assessment According to the Modified MINORS

The 7 eligible articles were evaluated according to the modified MINORS quality assessment. The methodological quality score of the included studies ranged from 14 to 22. All included studies were considered to be of moderate or high quality. Individual study characteristics, sterilization procedures, and quality assessment scores of all 7 included studies are summarized in Table 1.

The main disinfectants used in these 7 studies to decontaminate ACL grafts included a 4% chlorhexidine solution (173 samples in 5 studies), an antibiotic solution (158 samples in 5 studies), a 10% povidone-iodine solution (143 samples in 4 studies), saline (30 samples in 1 study), and a sodium hypochlorite solution (25 samples in 1 study). The

Figure 1. Flowchart describing the literature search and review process. ACL, anterior cruciate ligament.

| Lead Author (Year) | Country | Study Design  | Graft Type                                                                 | Modified MINORS Score |
|--------------------|---------|---------------|---------------------------------------------------------------------------|-----------------------|
| Molina19 (2000)    | USA     | Controlled trial | ACL specimens removed from patients undergoing total knee arthroplasty    | 16                    |
| Plante24 (2013)    | USA     | Controlled trial | Hamstring tendons harvested from patients                                 | 14                    |
| Badran2 (2016)     | Egypt   | Controlled trial | Hamstring tendon autograft specimens                                       | 19                    |
| Parker21 (2008)    | USA     | Controlled trial | Fresh-frozen bone–patellar tendon–bone grafts from screened donors        | 21                    |
| Cooper10 (1991)    | USA     | Controlled trial | Bone–patellar tendon–bone grafts harvested from fresh-frozen cadaveric knees | 16                    |
| Barbier4 (2015)    | France  | Controlled trial | Hamstring tendon autografts from patients                                  | 22                    |
| Goebel12 (1994)    | USA     | Controlled trial | Bone–patellar tendon–bone grafts harvested from adult California White rabbits | 16                    |

*aACL, anterior cruciate ligament; MINORS, Methodological Index for Non-Randomized Studies.*
decontamination methods included immersion for a variable duration of time, mechanical agitation and serial dilution, pulsed lavage, and a soak/rinse combination. Additionally, 6 studies\cite{2,4,10,12,19,24} reported the incidence of positive cultures of microbes after disinfecting the dropped ACL grafts with different disinfectants, while 1 study\cite{21} reported the number of colony-forming units after culturing. The ACL grafts in 1 study\cite{12} were harvested from rabbits, while the grafts used in the rest of the studies (2,4,10,19,21,24) were from humans. All 7 of these studies mainly evaluated the sterilization efficiency of different disinfectants on contaminated ACL grafts (Appendix Table A1).

**Culture Outcomes**

Overall, 5 studies\cite{2,4,10,19,24} reported the culture results of ACL grafts that fell on the operating room floor and remained there for 15 seconds or 3 minutes. The rate of contamination ranged from 23.3\% to 60.0\%, and the pooled rate of positive cultures was 44.9\% (92/205). The other 2 studies\cite{12,21} reported the simulation of microbial species and the number of microorganisms on the operating room floor based on the culture results. In these 7 studies, the ACL grafts dropped on the operating room floor were most commonly contaminated with staphylococci (Staphylococcus aureus and Staphylococcus epidermidis) and bacilli (Appendix Table A1). The contaminated ACL grafts treated with the antibiotic solution were mainly positive for staphylococci, bacilli, Clostridium, Acinetobacter, and diphtheroids. Simultaneously, staphylococci and bacilli were present on ACL grafts treated with the 10\% povidone-iodine solution and staphylococci and gram-negative bacilli on grafts treated with the 4\% chlorhexidine solution. The pooled results showed that the positive culture rates of disinfected ACL grafts with the 4\% chlorhexidine solution, antibiotic solution, and 10\% povidone-iodine solution were 2.3\% (4/173), 10.8\% (17/158), and 21.0\% (30/143), respectively.

**Comparison of 4% Chlorhexidine Solution Versus Antibiotic Solution**

Among the 7 included studies, 4 studies\cite{2,12,19,24} compared the sterilization efficiency between the 4\% chlorhexidine solution and an antibiotic solution on ACL grafts dropped on the operating room floor. The pooled results indicated that there was a significant difference in the incidence of positive cultures between the 2 groups, with the 4\% chlorhexidine solution displaying superior decontamination in comparison with the antibiotic solution (OR, 0.17 [95\% CI, 0.05-0.57]; \(P = .004\)). Heterogeneity was low, with almost all variations in the effect size attributed to random sampling errors (\(I^2 = 45\%\); \(P = .14\)) (Figure 2).

**Comparison of 10% Povidone-Iodine Solution Versus Antibiotic Solution**

Only 3 small studies\cite{2,12,19} evaluated the decontamination efficiency between the 10\% povidone-iodine solution and antibiotic solution, but 1 of them\cite{12} was not assessed because it reported that both the 10\% povidone-iodine solution and the antibiotic solution were 100\% ineffective. There was no heterogeneity among the results of these 3 studies (\(I^2 = 0\%\); \(P = .97\)), and the results were analyzed through a fixed-effects model. The pooled results revealed that the antibiotic solution showed superior decontamination to the 10\% povidone-iodine solution (OR, 0.20 [95\% CI, 0.07-0.55]; \(P = .002\)) (Figure 3).

**Comparison of 4% Chlorhexidine Solution Versus 10% Povidone-Iodine Solution**

There were 4 studies\cite{2,4,12,19} that compared the decontamination efficiency between the 4\% chlorhexidine solution and 10\% povidone-iodine solution on ACL grafts dropped on the operating room floor. There was heterogeneity between the results of each study (\(I^2 = 66\%\); \(P = .03\)), and the results were analyzed through a random-effects model, which showed no statistical significance between the 2 groups (OR, 0.09 [95\% CI, 0.01-0.96]; \(P = .005\)). Based on the sensitivity analysis results, heterogeneity arose mainly from the Barbier et al\cite{4} study. After excluding this study, no heterogeneity was found (\(I^2 = 0\%\); \(P = .44\)). After excluding the Barbier et al\cite{4} study, the pooled results showed that there was a significant difference between the 2 groups (OR, 0.04 [95\% CI, 0.01-0.20]; \(P < .0001\)) (Figure 4).

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**Figure 2.** Forest plot depicting the sterilization efficiency of the 4\% chlorhexidine solution versus the antibiotic solution. M-H, Mantel-Haenszel.
DISCUSSION

The present systematic review and meta-analysis revealed 2 main findings. One, the rate of accidentally dropped ACL grafts that resulted in positive bacterial cultures was 44.9% and predominantly consisted of staphylococci and bacilli. Two, comparing the sterilization efficiency of 3 different disinfectants, the 4% chlorhexidine solution showed excellent decontamination on the dropped ACL grafts, with the lowest positive culture rate of only 2.3%, followed by the antibiotic solution of 10.8% and the 10% povidone-iodine solution of 21.0%.

The inadvertent contamination of ACL grafts during ACL reconstruction has led to surgical complications, and there is currently no consensus in the literature to support the most appropriate management.\textsuperscript{13} If ACL grafts are dropped onto the operating room floor, proper and effective disinfection methods should be used to ensure low incidence of complications and the preservation of ACL grafts. Previous studies have reported some differences in the contamination rates of bone autografts intentionally dropped onto the operating room floor. Alomar et al\textsuperscript{1} conducted a laboratory study in which 69 fresh osteochondral autografts were dropped onto the operating room floor, and they found that the rate of contamination was 42%; the most common microorganisms were \textit{Staphylococcus epidermidis} (24.1%) and bacilli (20.7%). As part of an experiment, Hirn et al\textsuperscript{15} rubbed 60 femoral head specimens on the operating room floor and found coagulase-negative staphylococci and bacilli as the most prevalent microorganisms. In another study, Bruce et al\textsuperscript{7} found that the contamination rate of dropped osteo-articular fragments was 70%, with coagulase-negative staphylococci as prevalent microorganisms.

The present meta-analysis revealed that the contamination rate of grafts was 44.9%, and the 2 most common microorganisms identified from the dropped ACL grafts were staphylococci and bacilli, which is consistent with previously reported operating room microbial profiles.\textsuperscript{31} Similar to this study's results, Barbier et al\textsuperscript{4} reported that the risk of contamination of a hamstring graft dropped on the floor was up to 40%. However, in a previous experimental study, Burd et al\textsuperscript{8} deliberately dropped tendon grafts on the operating room floor and found that the contamination rate was variable. They reported that the contamination rate was 60% for bone and tendons, while it was about 10% for bone alone. This reminds us that the susceptibility to contamination of bone fragments and ACL grafts is different, and dropped bone fragments may be prone to a higher risk of contamination. Nevertheless, we should interpret these results cautiously, as positive bacterial culture results do not necessarily indicate septic arthritis after ACL

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Forest plot depicting the sterilization efficiency of the 10% povidone-iodine solution versus the antibiotic solution. M-H, Mantel-Haenszel.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Forest plot depicting the sterilization efficiency of the 4% chlorhexidine solution versus the 10% povidone-iodine solution. M-H, Mantel-Haenszel.}
\end{figure}
reconstruction in clinical practice. Also, Hantes et al\textsuperscript{14} conducted a prospective study and found no association between graft contamination and postoperative infection.

Although several experimental studies have investigated the management of ACL graft contamination,\textsuperscript{14,21,24} to our knowledge, there is no meta-analysis that has comprehensively reviewed these studies to date. In existing studies, disinfectants available in the operating room, including a chlorhexidine solution, antibiotic solution, and iodophor solution of variable concentrations, are usually the first choices. For instance, in 2011, Bauer et al\textsuperscript{6} conducted a study to compare the effectiveness of 5 different disinfectant methods on experimentally contaminated bone grafts. Although they proved that both dry iodophor and chlorhexidine produced excellent sterilization effects, the latter killed all live cells within the bone graft. Similarly, Bruce et al\textsuperscript{7} demonstrated that povidone-iodine and 4\% chlorhexidine gluconate were the most effective disinfectants for dropped osteoarticular bone fragments, but they did not recommend 4\% chlorhexidine gluconate as an effective decontaminant because it decreased chondrocyte cell viability. Liu et al\textsuperscript{18} found that the use of 2\% chlorhexidine significantly reduced the cell viability of osteoblasts, fibroblasts, and myoblasts and permanently stopped cell migration regardless of exposure time. It seems that chlorhexidine can kill the living cells of a bone graft, but its effect on ACL grafts is unclear, and it is essential to guide graft sterilization clinically.

Chlorhexidine belongs to the bisbiguanide class of antiseptics, which is cytotoxic to fibroblasts and negatively affects cell proliferation. Previous studies\textsuperscript{13,28} have shown that chlorhexidine does not affect the biological structure of tendon grafts. This effect on tendon grafts may be caused by the difference in porosity in which bone porosity allows easy ingress of detergents to the interior of bone. Further research is needed in the future to clarify the possible effects of these disinfectants on viable tendon cells. Also, it is important to note that no viable cells remain after the incorporation of typical ACL grafts.

There is only 1 systematic review that has comprehensively discussed the treatment strategies for intraoperative ACL graft contamination,\textsuperscript{17} and its authors noted that they believed that chlorhexidine is the best disinfectant for dropped tendon grafts. However, their investigation included different concentrations of detergents or sterilization techniques, and thus, there were no comparable data available to perform a meaningful meta-analysis. An antibiotic solution and povidone-iodine (10\% solution) are other common and easily available disinfectants. A povidone-iodine (10\% solution) is an effective antimicrobial remedy generally used for various purposes, including prophylactic disinfection and wound irrigation. Soyer et al\textsuperscript{29} recommended using a 10\% povidone-iodine solution to sterilize bone grafts but indicated that there must be sufficient exposure time according to the degree of contamination. However, Stanford et al\textsuperscript{30} demonstrated that the exposure of contaminated grafts to a 10\% povidone-iodine solution for 30 minutes resulted in incomplete sterilization.

In the present study, the dropped ACL grafts treated with the antibiotic solution had a 10.8\% positive culture rate, which showed a better disinfection effect than the 10\% povidone-iodine solution. Similarly, Yaman et al\textsuperscript{34} demonstrated that keeping contaminated graft materials in an antibiotic solution for specific periods yielded favorable outcomes. However, the ingredients of the antibiotic solutions included in the present study are not consistent; they consisted mainly of gentamicin, clindamycin, polymyxin, and bacitracin. Although vancomycin was proven to be an effective antibiotic for disinfecting ACL grafts,\textsuperscript{22,23} none of the studies included vancomycin. Schüttler et al\textsuperscript{27} used a porcine tendon model and demonstrated that 100\% of the tendon contaminated by \textit{Staphylococcus epidermidis} was effectively disinfected with vancomycin after 20 minutes. Perez-Prieto et al\textsuperscript{32} performed a prospective controlled study and found that ACL graft harvest and manipulation caused bacterial contamination in 14\% of cases but that contamination can be eliminated entirely after a soak in a vancomycin solution. Consistent with Perez-Prieto et al, another controlled observational study with 1585 patients by Phegan et al\textsuperscript{23} concluded that pre-soaking of hamstring grafts with topical vancomycin during ACL reconstruction might reduce the postoperative infection rate. However, Deijkers et al\textsuperscript{11} believed that irrigating contaminated grafts with an antibiotic solution had no benefit because the exposure time was not sufficient enough to make antibiotics effective.

Although the pooled results of the studies in the present meta-analysis showed that the antibiotic solution had an inferior sterilization effect on ACL grafts, we speculate that the difference is because of the inconsistent sensitivity of different microorganisms to antibiotics. Therefore, we believe that antibiotics sensitive to staphylococci and bacilli may be more suitable for the disinfection of dropped ACL grafts. Of course, further research should focus more on comparing the decontamination efficiency of vancomycin, 4\% chlorhexidine, and 10\% povidone-iodine.

Limitations

There are several limitations to this systematic review and meta-analysis. First, there was little uniformity across all studies in reporting cleaning methods after ACL grafts were contaminated. Additional procedures were performed in all the included studies, and thus, the effect of cleaning methods such as mechanical washing and rinsing could not be assessed. Second, the graft choice was variable among the included studies: A hamstring tendon autograft was used in 3 studies, while a bone–patellar tendon–bone graft was used in 3 other studies and ACL specimens in 1 more study. It has been proven that different grafts have different mechanical properties and clinical prognoses.\textsuperscript{9,26} However, in the present meta-analysis, we considered that the varied graft choice might have a negligible effect on the contamination event caused by the graft’s falling on the operating room floor. Moreover, we should also acknowledge the presence of bone in bone–patellar tendon–bone grafts.

A third limitation is that the components of the antibiotic solutions among the included studies were varied, and this aggregation may overlook some important information.
That said, it is challenging to collect efficient data because of the large difference in antibiotics involved in the studies, and no studies used vancomycin, which has been shown to be effective in reducing ACL infection rates. Fourth, the endpoint of the included studies was positive culture growth. However, it is noteworthy that positive culture endpoint of the included studies was positive culture of future infections. The present systematic review and meta-analysis results did not address the long-term infection risk associated with reimplanting contaminated grafts in humans, we believe that the culture-positive growth from the surgical environment could be considered a reasonable indicator for the risk of future infections.

CONCLUSION

The present systematic review and meta-analysis results demonstrated that the contamination of dropped ACL grafts during ACL reconstruction occurred at a relatively high rate, with staphylococci and bacilli being the most common microorganisms in dropped ACL grafts. Decontamination using a 4% chlorhexidine solution reliably disinfected ACL grafts that dropped on the operating room floor, with a disinfection rate of 97.7%. Based on the current data, we recommend disinfecting with a 4% chlorhexidine solution and reimplanting ACL grafts. This information can guide sports medicine surgeons to decide on appropriate remedial measures in similar dilemmas.

REFERENCES

1. Alomar AZ, Somly AM, Alraies TM, Bin Nasser AS, Ajlassir FF. Quantification analysis of the intraoperative bacterial contamination rate and level in osteochondral autografts. Am J Sports Med. 2016;44(3):761-766.
2. Badran MA, Moemen DM. Hamstring graft bacterial contamination during anterior cruciate ligament reconstruction: clinical and microbiological study. Int Orthop. 2016;40(9):1899-1903.
3. Bansal A, Lamplot JD, VandenBerg J, Brophy RH. Meta-analysis of the risk of infections after anterior cruciate ligament reconstruction by graft type. Am J Sports Med. 2018;46(6):1500-1508.
4. Barbier O, Danis J, Versier G, Ollat D. When the tendon autograft is contaminated using a 4% chlorhexidine solution and antiseptic efficacy. Knee. 2015;22(3):380-383.
5. Barriga A, Diaz-de-Rada P, Barroso JL, et al. Frozen cancellous bone allografts: positive cultures of implanted grafts in posterior fusions of the spine. Eur Spine J. 2004;13(2):152-156.
6. Bauer J, Liu RW, Kean TJ, Dennis JE, Petersilge W, Gilmore A. A comparison of five treatment protocols for contaminated bone grafts in reference to sterility and cell viability. J Bone Joint Surg Am. 2011;93(5):439-444.
7. Bruce B, Sheibani-Rad S, Appleyard D, et al. Are dropped ostearticular bone fragments safely reimplantable in vivo? J Bone Joint Surg Am. 2011;93(5):430-438.
8. Burd T, Conroy BP, Meyer SC, Allen WC. The effects of chlorhexidine irrigation solution on contaminated bone-tendon allografts. Am J Sports Med. 2000;28(2):241-244.
9. Chen W, Li H, Chen Y, Jiang F, Wu Y, Chen S. Bone–patellar tendon–bone autografts versus hamstring autografts using the same suspensory fixations in ACL reconstruction: a systematic review and meta-analysis. Orthop J Sports Med. 2019;7(11):2325967119885314.
10. Cooper DE, Armacquet SP, Warren RF. Contaminated patellar tendon grafts: incidence of positive cultures and efficacy of an antibiotic solution soak. An in vitro study. Arthroscopy. 1991;7(3):272-274.
11. Deijkers RL, Bloem RM, Petit PL, Brand R, Vehmey SB, Veen MR. Contamination of bone allografts: analysis of incidence and predisposing factors. J Bone Joint Surg Br. 1997;79(1):161-166.
12. Goebel ME, Drez D Jr, Heck SB, Stoma MK. Contaminated rabbit patellar tendon grafts: in vivo analysis of disinfecting methods. Am J Sports Med. 1994;22(3):387-391.
13. Han Y, Giannitissos D, Duke K, Steffen T, Burman M. Biomechanical analysis of chlorhexidine power irrigation to disinfect contaminated anterior cruciate ligament grafts. Am J Sports Med. 2011;39(7):1528-1533.
14. Hantes ME, Basdekis GK, Varitimides SE, Giotikas D, Petinaki E, Malizos KN. Autograft contamination during preparation for anterior cruciate ligament reconstruction. J Bone Joint Surg Am. 2008;90(4):760-764.
15. Hirn MY, Salmela PM, Vuento RE. High-pressure saline washing of allografts reduces bacterial contamination. Acta Orthop Scand. 2001;72(1):83-85.
16. Izquierdo R Jr, Cadet ER, Bauer R, Starwood W, Levine WN, Ahmad CS. A survey of sports medicine specialists investigating the preferred management of contaminated anterior cruciate ligament grafts. Arthroscopy. 2005;21(11):1348-1353.
17. Khan M, Rothrauff BB, Meralli F, Musahl V, Peterson D, Ayeni OR. Management of the contaminated anterior cruciate ligament graft. Arthroscopy. 2014;30(2):236-244.
18. Liu JX, Werner J, Kirsch T, Zuckerman JD, Virk MS. Cytotoxicity evaluation of chlorhexidine gluconate on human fibroblasts, myoblasts, and osteoblasts. J Bone Joint Infect. 2018;3(4):165-172.
19. Molina ME, Nonweiller DE, Evans JA, Delee JC. Contaminated anterior cruciate ligament grafts: the efficacy of 3 sterilization agents. Arthroscopy. 2000;16(4):373-378.
20. Nagda SH, Altobelli GG, Bowdry KA, Brewster CE, Lombardo SJ. Cost analysis of outpatient anterior cruciate ligament reconstruction: autograft versus allograft. Clin Orthop Relat Res. 2010;468(5):1418-1422.
21. Parker RD, Maschke SD. Mechanical agitation and serial dilution: an option for anterior cruciate ligament graft sterilization. J Knee Surg. 2008;21(3):186-191.
22. Perez-Prieto D, Portillo ME, Torres-Claramunt R, Pelfort X, Hinarejos P, Monllau JC. Contamination occurs during ACL graft harvesting and manipulation, but it can be easily eradicated. Knee Surg Sports Traumatol Arthrosc. 2018;26(2):558-562.
23. Phegan M, Grayson JE, Vertullo CJ. No infections in 1300 anterior cruciate ligament reconstructions with vancomycin pre-soaking of hamstring grafts. Knee Surg Sports Traumatol Arthrosc. 2016;24(9):2729-2735.
24. Plante MJ, Li X, Scully G, Brown MA, Busconi BD, DeAngelis NA. Evaluation of sterilization methods following contamination of hamstring autograft during anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc. 2013;21(3):696-701.
25. Pogorzelski J, Themessl A, Achtinich A, et al. Septic arthritis after anterior cruciate ligament reconstruction: how important is graft salvage? Am J Sports Med. 2018;46(10):2376-2383.
26. Salem HS, Varzhapetyan V, Patel N, Dodson CC, Tjoumakaris FP, Freedman KB. Anterior cruciate ligament reconstruction in young female athletes: patellar versus hamstring tendon autografts. Am J Sports Med. 2019;47(9):2086-2092.
27. Schüttler KF, Schamm A, Stein T, et al. Biomechanical and microbial effects of local vancomycin in anterior cruciate ligament (ACL) reconstruction: a porcine tendon model. Arch Orthop Trauma Surg. 2019;139(1):73-78.
28. Sobel AD, Hohman D, Jones J, Bisson LJ. Chlorhexidine gluconate cleansing has no effect on the structural properties of human patellar tendon allografts. Arthroscopy. 2012;28(12):1862-1866.
29. Soyer J, Rouil M, Castel O. The effect of 10% povidone-iodine solution on contaminated bone allografts. J Hosp Infect. 2002;50(3):183-187.
30. Stanford R, Solomon M, Levick M, Kohan L, Bell S. Sterilization of contaminated bone-tendon autografts using 10% povidone-iodine solution. *Orthopaedics*. 1999;22(8):601-604.

31. Suzuki A, Namba Y, Matsuura M, Horisawa A. Bacterial contamination of floors and other surfaces in operating rooms: a five-year survey. *J Hgy (Lond)*. 1984;93(3):559-566.

32. Vertullo CJ, Quick M, Jones A, Grayson JE. A surgical technique using presoaked vancomycin hamstring grafts to decrease the risk of infection after anterior cruciate ligament reconstruction. *Arthroscopy*. 2012;28(3):337-342.

33. Wang C, Lee YH, Siebold R. Recommendations for the management of septic arthritis after ACL reconstruction. *Knee Surg Sports Traumatol Arthrosc*. 2014;22(9):2136-2144.

34. Yaman F, Unlu G, Atligan S, Celik Y, Ozekinci T, Yaldiz M. Microbiologic and histologic assessment of intentional bacterial contamination of bone grafts. *J Oral Maxillofac Surg*. 2007;65(8):1490-1494.

APPENDIX

TABLE A1
Disinfectants, Sterilization Methods, and Outcomes of Included Studies

| Lead Author (Year) | Contamination Method | Disinfectants | Cleaning Method | Outcome Evaluation | No. of Positive Cultures/Total: Types of Microorganisms |
|--------------------|----------------------|---------------|----------------|-------------------|---------------------------------------------------------|
| Molina19 (2000)   | Dropped on the floor for 15 s | Untreated, Antibiotic solution (40 mg of neomycin sulfate and 200,000 U of polymyxin B sulfate in 1000 mL of sterile saline), 10% povidone-iodine solution, 4% chlorhexidine solution | Immersion for 90 s | Culture | 29/50: *Staphylococcus*, *Bacillus* |
|                   |                      | Uncontaminated graft after harvest | Soak for 3 min | Culture | 3/50: *Bacillus*, *Clostridium* |
|                   |                      | Graft dropped on the floor for 5 s | Soak for 3 min | Culture | 12/50: *Bacillus*, *Staphylococcus* |
|                   |                      | Graft dropped on the floor for 15 s | Soak for 3 min | Culture | 1/50: gram-negative rods |
|                   |                      | Saline solution | Soak for 3 min | Culture | 7/30: *S aureus*, viridans streptococci |
|                   |                      | Bacitracin solution (50,000 U/L of normal saline) | Soak for 3 min | Culture | 10/30: *S aureus* |
|                   |                      | 4% chlorhexidine solution | Soak for 3 min | Culture | 9/30: *S aureus* |
|                   |                      | | Soak for 3 min | Culture | 1/30: nonaureus staphylococci |
|                   |                      | | Soak for 3 min | Culture | 1/30: *Bacillus* |
| Badran2 (2016)    | Dropped on the floor adjacent to the surgical field for 15 s | Untreated, 10% povidone-iodine solution, 4% chlorhexidine solution, Bacitracin solution (50,000 U/L of normal saline) | Immersion for 3 min | Culture | 30/60: *S epidermidis* |
|                   |                      | | Soak/rinse for 3 min | Culture | 9/60: *S epidermidis*, *S aureus* |
|                   |                      | | Soak/rinse for 3 min | Culture | 0/60: NG |
|                   |                      | | Soak/rinse for 15 min | Culture | 2/60: *S epidermidis*, *Acinetobacter* |
| Parker21 (2008)   | Determined bacterial flora on the operating room floor and contaminated in a bacterial suspension for 15 s | Untreated, Antibiotic solution (166.66 U/mL of polymyxin B and 16.66 U/mL of bacitracin), Pulsatile lavage with antibiotic solution, Mechanical agitation and serial dilution with antibiotic solution | Soak/pulsatile lavage/mechanical agitation and serial dilution for 15 min | Semiquantitative culture | 10/10 (81 CFU): *Staphylococcus*, *Bacillus* |
|                   |                      | | Immersion for 15 min | Culture | 10/10 (17.4 CFU): *Staphylococcus*, *Bacillus* |
|                   |                      | | Immersion for 15 min | Culture | 4/10 (0.6 CFU): *Staphylococcus*, *Bacillus* |
|                   |                      | | Soak/pulsatile lavage/mechanical agitation and serial dilution for 15 min | Semiquantitative culture | 0/10 (0 CFU): NG |
| Cooper10 (1991)   | Dropped on the operating room floor for 3 min | Untreated, Antibiotic solution (33.33 U/mL of bacitracin and 33.33 U/mL of polymyxin B) | Soak/rinse for 15 min | Culture | 6/10: diphtheroids, *Bacillus* |
|                   |                      | | Soak/rinse for 15 min | Culture | 3/10: *S epidermidis*, diphtheroids |
|                   |                      | | Soak/rinse for 15 min | Culture | 10/25: *Staphylococcus* |
|                   |                      | | Soak/rinse for 15 min | Culture | 2/25: *Aerococcus sanguinicola*, *S aureus*, *S epidermidis* |
|                   |                      | | Soak/rinse for 15 min | Culture | 1/25: *S capitis* |
|                   |                      | | Soak/rinse for 15 min | Culture | 4/25: *S hominis*, *S capitis*, *S warneri* |
| Barbiere4 (2015)  | Dropped on the operating room floor for 15 s | Untreated, 4% chlorhexidine gluconate solution, 10% povidone-iodine solution, 0.5 g/100 mL sodium hypochlorite solution | Immersion for 15 min | Culture | 6/6: coagulase-negative *staphylococci* |
|                   |                      | | Immersion for 15 min | Culture | 8/6: *Staphylococcus* |
|                   |                      | | Immersion for 15 min | Culture | 8/8: *S capitis*, coagulase-negative *staphylococci* |
|                   |                      | | Immersion for 15 min | Culture | 0/8: NG |

*CFU, colony-forming unit; NG, no growth.*