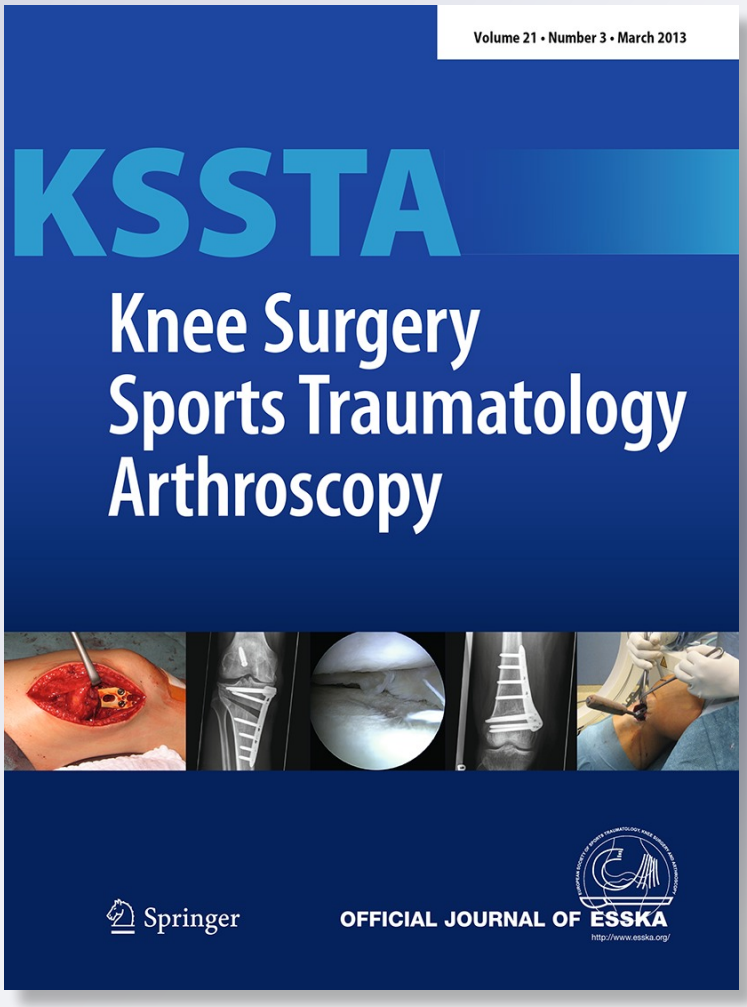
Evaluation of sterilization methods following contamination of hamstring autograft during anterior cruciate ligament reconstruction

Matthew J. Plante, Xinning Li, Gail Scully, Michael A. Brown, Brian D. Busconi & Nicola A. DeAngelis

Knee Surgery, Sports Traumatology, Arthroscopy

ISSN 0942-2056
Volume 21
Number 3

Knee Surg Sports Traumatol Arthrosc
(2013) 21:696-701
DOI 10.1007/s00167-012-2049-8



Springer

Knee Surg Sports Traumatol Arthrosc (2013) 21:696–701
DOI 10.1007/s00167-012-2049-8

KNEE

Evaluation of sterilization methods following contamination of hamstring autograft during anterior cruciate ligament reconstruction

Matthew J. Plante · Xinning Li · Gail Scully · Michael A. Brown · Brian D. Busconi · Nicola A. DeAngelis

Received: 25 February 2012 / Accepted: 2 May 2012 / Published online: 15 May 2012

Abstract
Purpose Inadvertent contamination of the hamstring autograft during ACL reconstruction is infrequent, but can result in significant complications. The purpose of this study is to evaluate bacterial contamination of hamstring autografts dropped onto the operating room floor and methods of graft decontamination.
Methods Hamstring tendons were harvested from patients. Excess tendon not used in the ACL procedure was divided into 6 segments. Segments were assigned to 6 groups (A through F, $N = 30$ in each group): group A: uncontaminated graft immediately postharvest (control), group B: graft dropped onto the floor (5 s), group C: graft dropped onto the floor (15 s). grafts in groups D to F were dropped onto floor for 15 s then rinsed with saline (group D), bacitracin solution (group E) or chlorhexidine 4 % solution (group F) for 3 min. All grafts were sent to the microbiology laboratory for anaerobic and aerobic cultures.
Results Cultures were positive in 23 % of graft segments from group A (7/30), 33 % of grafts from group B (10/30), 23 % from group C (7/30), 30 % from group D (9/30) and 3 % from both group E (1/30) and group F (1/30). Sixteen unique organisms were identified, with *Staphylococcus aureus* as the most common isolate. Grafts rinsed in either bacitracin solution or 4 % chlorhexidine solutions were significantly less likely to be culture positive when compared to control graft segments ($p < 0.05$). However, there was no significant difference between uncontaminated grafts retrieved in <5 versus 15 s from the floor.
Conclusion This study supports the practice of decontaminating a dropped ACL hamstring autograft using either 4 % chlorhexidine or bacitracin solution. Specimens should be retrieved sterilely and washed for at least 3 min. This study also demonstrates no advantage in retrieval time of less than 5 s as compared to 15 s for uncontaminated graft. Hamstring harvest in ACL reconstruction may result in positive cultures, thus routine soaking of the hamstring autograft in either bacitracin or 4 % chlorhexidine solution is recommended. In addition, dropped hamstring autograft can be effectively sterilized with bacitracin or 4 % chlorhexidine solution.
Level of evidence II.

Keywords ACL · Hamstring · Autograft · Infection · Sterilization

Introduction

The anterior cruciate ligament (ACL) is an important stabilizer of the knee and prevents anterior translation of the tibia on the femur. ACL rupture is a common sports-related injury in the young population with an annual incidence of approximately 250,000 occurrences [1]. Each year, over 200,000 arthroscopic-assisted ACL reconstructions are performed in the United States [7, 18]. Inadvertent contamination of the graft when performing ACL reconstruction is an infrequent but significant complication. Most commonly, this occurs when the graft is accidently dropped onto the operating room floor. Twenty-five per cent of all fellowship-trained sports medicine surgeons report

M. J. Plante · X. Li (✉) · G. Scully · M. A. Brown · B. D. Busconi · N. A. DeAngelis
Division of Sports Medicine, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA, USA
e-mail: Xinning.li@gmail.com

G. Scully
Division of Infectious Disease, University of Massachusetts Medical Center, 55 Lake Avenue North, Worcester, MA, USA

experiencing at least one such event [4]. Other studies have demonstrated that contamination rates of routine graft harvesting may be as high as 12 % [8, 10], despite no apparent contamination occurrence.

After graft contamination, a surgeon faces a dilemma. Potential options include harvesting another type of graft, obtaining the graft from the contralateral limb, switching to allograft tissue or attempting to decontaminate and sterilize the graft. Harvesting another graft exposes the patient to added donor site morbidity and increased risk of infection. Utilizing allograft tissue adds considerable cost to the procedure [14], increases the risk of disease transmission, delayed incorporation, risk of tunnel enlargement, question of availability and possible compromise in functional outcomes [12]. Proceeding by using the contaminated graft raises obvious concerns of increased infection risk. A survey of sports medicine specialists raised the question of how to manage an intraoperative graft contamination when performing ACL reconstruction [11]. The most frequent response (75 %) was an attempt to decontaminate the graft and proceed with the operation. Less frequently selected options included harvesting another type of autograft or switching to allograft tissue. While other studies have investigated the effectiveness of decontamination of various autologous and allograft tissue [2, 4, 13, 17], no studies have specifically examined the decontamination of hamstring autograft tissue.

The purpose of this study is to evaluate bacterial contamination of hamstring autografts dropped onto the operating room floor and to determine whether these grafts may be adequately sterilized with various cleansing techniques available in the operating room. The hypothesis is that both the 4 % chlorhexidine and bacitracin solution will effectively sterilize the dropped hamstring autografts in comparison to normal saline solution.

Materials and methods

This study was submitted for IRB review at the University of Massachusetts Medical Center and granted exemption. Thirty consecutive patients undergoing hamstring tendon autograft ACL reconstruction were consented and included in this study. When necessary, the surgical area was shaved preoperatively. Skin preparation was performed using ChloraPrep® (2 % chlorhexidine gluconate and 70 % isopropyl alcohol). All patients were given routine single-dose antibiotic (second-generation cephalosporin unless allergic) prior to incision.

Semitendinosis and gracilis tendons were harvested using standard technique. Muscle was then removed from the tendons. Both tendons were then cut to a length of 22 cm, and a quadruple-stranded ACL graft was constructed. The excess tendon tissue was then taken to a sterile side table and divided into six segments (ranging in size from 0.8 cm to 1.6 cm depending on the length of tendon harvested). The segments were labelled A thru F. One uncontaminated segment was sent for culture as a control (A). The second segment (B) was dropped onto the floor adjacent to the surgical field, immediately retrieved (in less than 5 s) using sterile forceps, and sent for culture. The remaining four segments were dropped onto the floor adjacent to the surgical field for fifteen seconds. One segment was then cultured after being retrieved from the floor without undergoing any further treatment (C). The remaining three segments were soaked in normal saline (D), 4 % chlorhexidine (E) or bacitracin (50,000 units per 1 L normal saline) antibiotic solution (F), respectively, for 3 min and then cultured. The floor was then swabbed at the site where the specimens were dropped and that was also sent for culture (G).

Statistical analysis

Statistical analysis was performed using the two-sample test of proportions for a binomial distribution. A two-tailed p value was calculated. See Table 1 for the full description of each experimental condition and the p values.

Results

Positive cultures were seen (Table 2) in the control group (7/30), group B (10/30), group C (7/30), group D (9/30), group E (1/30) and group F (1/30). Sixteen unique organisms were identified (Fig. 1) with *Staphylococcus aureus* being the most common. Overall, a total of 75 isolates were identified by culture with *S. Aureus* representing 44 %, *coagulase negative Staphylococcus* 9.3 %, *Streptococcus viridians* 5.3 %, *Corynebacterium* species 6.7 %, *Propionibacterium acnes* 10.7 %, *Lactobacillus* species 1.3 %, *Escherichia coli* 1.3 %, *Prevotella buccae* 1.3 %, *Citrobacter freundii* 1.3 %, *Pseudomonas aeruginosa* 2.7 %, *Bacillus* species 9.3 %, *Sphingomonas paucimobilis* 1.3 %, *Moraxella* 1.3 %, *Clostridium Sordelli* 1.3 %, *Escherichia hermannii* 1.3 % and *Stenotrophomonas maltophilia* 1.3 %.

In group B that was dropped onto the floor and retrieved within 5 s, there were 10 positive cultures (33 %) and 20 negative cultures (67 %). In the 10 positive cultures, 6 different organisms were identified. In the group C that was dropped onto the floor and retrieved at 15 s and did not undergo decontamination, 7 cultures were positive (23 %) and 23 were negative (77 %). In this particular group, 6 different organisms were identified. Statistical analysis was performed using the two-sample test of proportions for a

Table 1 Full description of each experimental condition performed in this study

Specimen	Type	Description
A	Hamstring tissue	Immediately after harvest
B	Hamstring tissue	On floor 5 s
C	Hamstring tissue	On floor 15 s
D	Hamstring tissue	On floor 15 s, 3 min saline wash
E	Hamstring tissue	On floor 15 s, 3 min 4 % chlorhexidine wash
F	Hamstring tissue	On floor 15 s, 3 min antibiotic solution wash
G	Culture swab	Swab of floor adjacent to operative field

binomial distribution. A two-tailed p-value was calculated. (See Table 2). There was no significant difference in culture positivity between the two contaminated ACL groups of 5 s versus 15 s of floor contact (p = n.s.). In the uncontaminated ACL group (A), 7 out of 30 cultures (23 %) were positive with 7 organisms identified. There was also no significant difference in culture positivity between the uncontaminated ACL graft and graft that had been dropped onto the floor (5 vs. 15 s time).

In groups D, E and F, all grafts had 15 s of floor contact prior to rinsing in normal saline (D), chlorahexadine 4 % (E) or bacitracin solution (F) for 3 min. In group D rinsed in normal saline, 9 out of 30 cultures were positive (30 %) with 5 organisms identified. Groups E and F rinsed in either chlorhexidine 4 % or bacitracin solution for 3 min had only 1 out 30 cultures (3 %) that were positive. A statistically significant difference was noted between the control ACL group and the two ACL groups rinsed in chlorhexidine or bacitracin solution ($p < 0.05$). A significant difference was also seen between the control, uncontamined ACL tendon and a swab culture taken directly from the floor ($p < 0.05$). Sixty-three per cent of cultures taken from the floor were positive versus 23 % positive cultures in the control ACL group.

Discussion

Anterior cruciate ligament reconstruction is a commonly performed procedure in the United States. In a survey of sports medicine fellowship–trained physicians, 25 % (49/196) have encountered graft contamination during the intraoperative setting. Several options are available when this occurs. In the survey, 75 % of the surgeons in this situation cleansed and implanted the dropped graft, 18 % used alternative autograft options including the contralateral limb and 7 % used allograft [11]. While other studies have investigated the effectiveness of decontamination of various autologous and allograft tissues, no studies have specifically examined the decontamination of hamstring autograft tissue. To our knowledge, this is the first study to evaluate ACL graft contamination and decontamination using actual autograft hamstring tissue in the operating room setting. Molina et al. [13] found 4 % chlorhexidine and double antibiotic solution (neomycin and polymyxin B) successfully decontaminated dropped native ACLs at a rate of 98 and 94 %, respectively. However, when providone–iodine solution was used, 24 % of the ACL graft had resulted in positive cultures. Other studies have evaluated the effectiveness of decontamination on allograft tissue [2, 17]. This study was designed to mimic an actual intraoperative graft contamination event, specifically a hamstring autograft falling to the floor, to determine whether the tissue can be safely decontaminated. To accomplish this, the tissue was dropped onto the floor during the actual ACL surgery at a spot located between the graft prep table and the operative field. We also left the graft on the floor for both 5 and 15 s to simulate actual intraoperative time for which the graft would be retrieved from the floor.

An antibiotic solution (bacitracin) and 4 % chlorhexidine were chosen as the decontamination agents for this study as they have proven effective in other decontamination studies [2, 13]. Betadine was not evaluated as part of our study, as the literature suggests it is inferior to both antibiotic solution and chlorhexidine in terms of decontaminating tissue [4, 13]. This study sought to determine whether the results from these other studies could be reproduced when applied to actual hamstring autograft tissue. In this study, there was only one positive culture (3 %) for both the bacitracin solution and 4 % chlorhexidine group. The contaminated grafts were washed for a total of 3 min, which does not cause a significant delay to the overall surgical procedure. This provides further evidence that dropped ACL hamstring autografts can be safely decontaminated in a reasonably short period of time using agents readily available in most operating rooms. Utilizing antibiotic solution to sterilize autografts dropped onto the floor has also been reported in the literature as clinical case reports. Casalonga et al. [3] followed the outcome of four patients in whom the B-T-B graft dropped onto the floor was re-implanted after decontamination with topic antibiotics. The grafts were soaked in rifamycin and then

Table 2 Bacterial culture results of each experimental condition

Specific conditions	Positive cultures	Bacteria types	Negative cultures	Percentage positive culture	*p* value
Send from body	7	*Staph. aureus* (3) *Staph.* non *aureus* (1) *Strept. Viridians* (3) *Corynebacterium* (2) *Lactobacillus* (1) *P. acnes* (1) *E. Coli* (1)	23	7/30 = 23 %	n.s
Dropped onto the floor (5 s)	10	*Staph. aureus* (7) *Corynebacterium* (1) *P. buccae* (1) *P. acnes* (1) *C. freundii* (1)	20	10/30 = 33 %	n.s
Dropped onto the floor (15 s)	7	*Staph. aureus* (3) *Staph.* non *aureus* (3) *P. acnes* (2) *Corynebacterium* (1) *P. aeruginosa* (1)	23	7/30 = 23 %	Control
Dropped onto the floor (15 s) Rinsed in saline (3 min)	9	*Staph. aureus* (5) *Staph.* non *aureus* (1) *P. acnes* (2) *P. Aeruginosa* (1) *S. paucimobilis* (1)	21	9/30 = 30 %	n.s
Dropped onto the floor (15 s) Rinsed in Chlorahexadine (4 %) (3 min)	1	*Bacillus* (1)	29	1/30 = 3 %	0.03
Dropped onto the floor (15 s) Rinsed in antibiotics solution (3 min)	1	*Staph.* non *aureus* (1)	29	1/30 = 3 %	0.03
Swab on floor in area where graft was dropped	19	*Staph. aureus* (14) *Staph.* non *aureus* (1) *Strept. viridians* (1) *Corynebacterium* (1) *P. acnes* (2) *Bacillus* (6) *Moraxella* (1) *Pseudomonas alcaligenes* (1) *C. sordelli* (1) *E. hermannii* (1) *S. maltophilia* (1)	11	19/30 = 63 %	0.002

n.s, non-significant

gentamycin for 10 min each along with postoperative antibiotics for 15 days. There were no complications or postoperative infections, and all patients were able to return to previous sport level. Pasque et al. [16] also reported 3 cases where the graft was dropped onto the floor after harvest. They utilized a protocol of removing all suture material, rinsing in chlorhexidine solution for 15 min, then saline for 15 min and 10 days of postoperative antibiotics. All patients had uneventful recovery without evidence of clinical infection.

In this study, inadvertent contamination was found in (23 %) of specimens sent directly to laboratory following harvesting. However, none of these patients developed any clinical infection postoperatively. A similar phenomenon has been described elsewhere [8, 10]. Hantes et al. [10] found a high overall rate of autograft contamination (12 %)

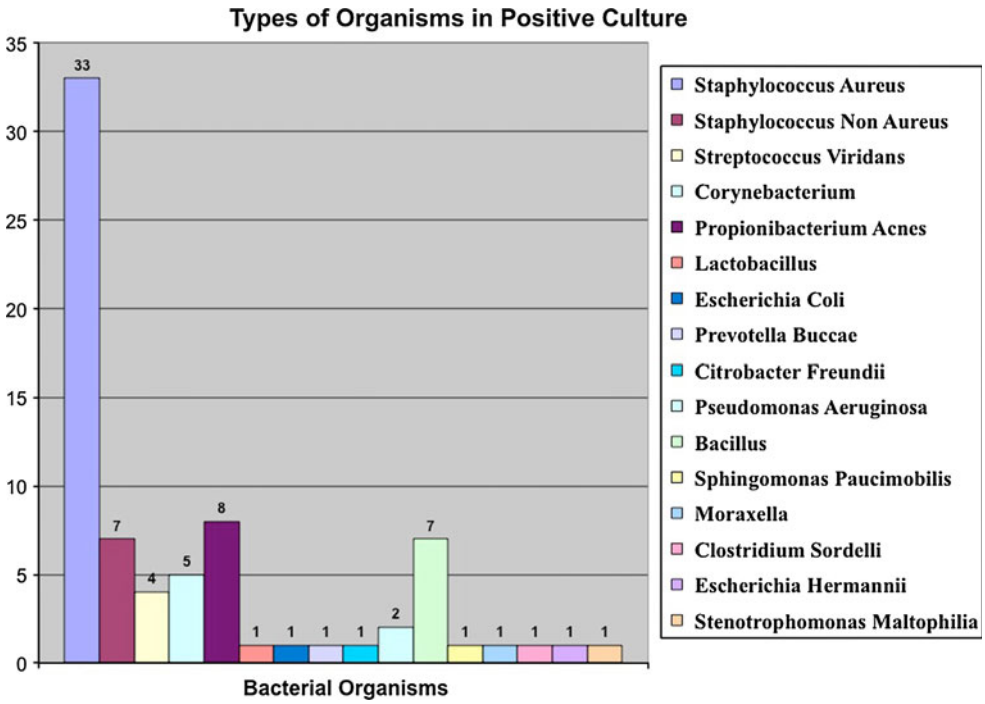


Fig. 1 All of the organisms seen in the positive cultures

during preparation for ACL reconstruction with similar rates between bone-patella-bone (B-T-B) and hamstring. They initially hypothesized that the increased length of time for hamstring graft preparation in comparison to B-T-B graft would have resulted in a significantly higher contamination rate for hamstring grafts; however, the authors concluded that their study was underpowered to detect any real significant difference between the two groups. Another possible source of contamination of autograft harvest could be due to inadequate sterilization of tendon harvesters [19]. Gavriilidis et al. [8] reported a 10 % rate of positive cultures in 89 hamstring autografts harvested for ACL reconstruction. Positive cultures after implantation of allograft tissue for ACL reconstruction have been reported in 9.7 to 13.3 % of specimens [6, 9]. Similar to our study, none of the patients in these four studies had developed evidence of clinical infection. This may be due to the protective effect of preoperative intravenous antibiotics.

Fifteen seconds was chosen as the amount of time for the tissue to remain on the floor, as this is a reasonable amount of time for the graft to be sterilely retrieved in a real operating room environment. Immediate graft retrieval ("the 5-s rule") did not affect the rate of contamination when compared to fifteen-second exposure (33 vs. 23 %, p = n.s.). Longer contamination exposures were not evaluated as this was not felt to be realistic in the operating room setting. The time on the floor does not seem to affect the rate of positive cultures, and this is further supported in the literature. Molina et al. [13] reported a 58 % positive culture rate when the ACL specimens were dropped onto the floor for 15 s in comparison to Cooper et al. [5], which had a similar infection rate of 60 % after the graft was on the floor for 3 min. However, the disparity between positive floor culture swabs (63 %) and untreated contaminated graft specimens (on floor for 15 s) (23 %) is not easily explained as the floor swabs were taken from the location where the specimens were dropped. Moline et al. [13] noted a similar finding in their study of contaminated native ACLs. This discrepancy may also be caused by the protective effect from routine preoperative antibiotic administration.

Overall, a total of 75 isolates were identified by positive culture, and the most common organisms were *S. Aureus* (44 %), *P. acnes* (10.7 %), coagulase negative *Staphylococcus* (9.3 %) and *Bacillus* (9.3 %). (See Table 2 for complete listing of all organisms identified from culture.) In the group rinsed in chlorhexidine solution, the only positive culture was *Bacillus* species, and in the group rinsed with bacitracin antibiotics solution, the positive culture was coagulase-negative *Staphylococcus*. Molina et al. [13] reported similar organisms seen in their study with *S. aureus*, coagulase-negative *Staphylococcus* and *Bacillus* being the most common. In their study, *Bacillus* and *Clostridium* species were the only organisms seen after neomycin and polymyxin B rinse, and a gram-negative rod was identified in one culture after chlorhexidine rinse. However, they reported 24 % positive cultures after 10 % providone–iodine solution rinse with majority of the organisms being *Staphylococcus* and *bacillus* species.

One limitation of this study is that the hamstring tissue evaluated did not contain any suture material. Depending

in what step of the case contamination occurs, a dropped graft may include braided suture, which could potentially become contaminated as well. This study does not address whether suture material can be effectively decontaminated. It is therefore the recommendation of the authors that all suture material be removed from the graft prior to attempting to decontaminate. Furthermore, our study had a low rate of positive cultures (23–33 %) from grafts that were dropped onto the floor. One possible explanation for this low rate is the small segment (0.8–1.6 cm) that was dropped onto the floor had less surface area exposed for contamination. Furthermore, the graft was left in the same place on the floor and picked up with sterile forceps before taken to the microbiology laboratory. We believe this simulates realistic intraoperative steps in the event of a dropped graft. If each graft was moved around on the floor and picked up with non-sterile equipment, then our positive culture results would have been higher. In addition, our operating room floors were cleaned with bleach between each case; this may also contribute to the lower rate of the positive cultures from the floor. Another limitation is that no histological or biomechanical evaluation was performed on the hamstring tissue following decontamination. Further studies are needed to evaluate the effect of antibiotic solution or 4 % chlorhexidine on the structural integrity of hamstring tissue. It is interesting to note that many surgeons routinely soak hamstring autografts in antibiotic solution prior to implantation. This additional step may further decrease the risk of infection after hamstring ACL reconstruction, given that 23 % of uncontaminated native graft from the patient's body sent to the microbiology laboratory had positive cultures. However, soaking autografts in antibiotic solution routinely may increase the risk of producing multi-resistent organisms. Alternatively, Parker et al. [15] found that mechanical agitation and serial dilution were also very effective in sterilizing contaminated ACL bone-patella-bone grafts, which resulted in zero colony-forming units with culture.

Conclusion

This study supports the practice of decontaminating a dropped ACL hamstring autograft that became contaminated after inadvertent floor contact using either 4 % chlorhexidine or a bacitracin antibiotic solution (50,000 units/1L NS). Specimens should be retrieved sterilely and washed for at least 3 min. This study demonstrates no advantage to retrieval time of 5 versus 15 s.

Acknowledgments Funding for this study was provided internally by the University of Massachusetts Medical Center, Worcester, MA.

References

1. Boni DM, Herriott GE (2002) Hamstring tendon graft for anterior cruciate ligament reconstruction. AORN J 76(4):610–615, 7–9, 21–24; quiz 25–28
2. Burd T, Conroy BP, Meyer SC et al (2000) The effects of chlorhexidine irrigation solution on contaminated bone-tendon allografts. Am J Sports Med 28(2):241–244
3. Casalonga D, Ait Si Selmi T, Robinson A et al (1999) Peroperative accidental contamination of bone-tendon-bone graft for the reconstruction of the anterior cruciate ligament. Report of 4 cases. Rev Chir Orthop Reparatrice Appar Mot 85(7):740–743
4. Centeno RF, Desai AR, Watson ME (2008) Management of contaminated autologous grafts in plastic surgery. Eplasty 8:e23
5. Cooper DE, Arnoczky SP, Warren RF (1991) Contaminated patellar tendon grafts: incidence of positive cultures and efficacy of an antibiotic solution soak—an in vitro study. Arthroscopy 7(3):272–274
6. Diaz-de-Rada P, Barriga A, Barroso JL et al (2003) Positive culture in allograft ACL-reconstruction: what to do? Knee Surg Sports Traumatol Arthrosc 11(4):219–222
7. Freedman KB, D'Amato MJ, Nedeff DD et al (2003) Arthroscopic anterior cruciate ligament reconstruction: a metaanalysis comparing patellar tendon and hamstring tendon autografts. Am J Sports Med 31(1):2–11
8. Gavriilidis I, Pakos EE, Wipfler B et al (2009) Intra-operative hamstring tendon graft contamination in anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 17(9):1043–1047
9. Guelich DR, Lowe WR, Wilson B (2007) The routine culture of allograft tissue in anterior cruciate ligament reconstruction. Am J Sports Med 35(9):1495–1499
10. Hantes ME, Basdekis GK, Varitimidis SE et al (2008) Autograft contamination during preparation for anterior cruciate ligament reconstruction. J Bone Joint Surg Am 90(4):760–764
11. Izquierdo R Jr, Cadet ER, Bauer R et al (2005) A survey of sports medicine specialists investigating the preferred management of contaminated anterior cruciate ligament grafts. Arthroscopy 21(11):1348–1353
12. Marrale J, Morrissey MC, Haddad FS (2007) A literature review of autograft and allograft anterior cruciate ligament reconstruction. Knee Surg Sports Traumatol Arthrosc 15(6):690–704
13. Molina ME, Nonweiller DE, Evans JA et al (2000) Contaminated anterior cruciate ligament grafts: the efficacy of 3 sterilization agents. Arthroscopy 16(4):373–378
14. Nagda SH, Altobelli GG, Bowdry KA et al (2009) Cost analysis of outpatient anterior cruciate ligament reconstruction: autograft versus allograft. Clin Orthop Relat Res 468:1418–1422
15. Parker RD, Maschke SD (2008) Mechanical agitation and serial dilution: anoption for anterior cruciate ligament sterilzation. J Knee Surg 21(3):186–191
16. Pasque CB, Geib TM (2007) Intraoperative anterior cruciate ligament graft contamination. Arthroscopy 23(3):329–331
17. Saegeman VS, Ectors NL, Lismont D et al (2009) Effectiveness of antibiotics and antiseptics on coagulase-negative staphylococci for the decontamination of bone allografts. Eur J Clin Microbiol Infect Dis 28(7):813–816
18. Shelton WR, Fagan BC (2011) Autografts commonly used in anterior cruciate ligament reconstruction. J Am Acad Orthop Surg 19(5):259–264
19. Tuman J, Diduch DR, Baumfeld JA et al (2008) Joint infection unique to hamstring tendon harvester used during anterior cruciate ligament reconstruction surgery. Arthroscopy 24(5):618–620