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Hamstring autografts are associated with a high rate of contamination in anterior cruciate ligament reconstruction

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Abstract
Purpose To quantitatively evaluate the rate, type, and level of contamination of anterior cruciate ligament (ACL) hamstring autografts after harvesting and preparation or dropping onto the operating room (OR) floor.
Methods Two hamstring autograft specimens were prospectively retrieved from each graft in a consecutive series of 50 patients undergoing primary isolated ACL reconstruction (100 specimens total). One specimen was retrieved immediately after harvesting and dropped onto the OR floor (dropped group). The other was retrieved just after graft implantation and before fixation (control group). Each specimen was incubated for aerobic and anaerobic growth, and the number of colony-forming units (CFU)/g was measured. Patients' clinical course was monitored for signs of surgical site infection (SSI).
Results The control and dropped groups had positive culture rates of 11/50 (22%) and 16/50 (32%), respectively, with no significant difference between groups (n.s.). The most common organism in the control group was *Staphylococcus epidermidis* (45.5%) followed by *S. aureus* (36.4%). In the dropped group, the most common organism was *S. epidermidis* (31.3%) followed by *Bacillus* species (25%). The median (range) CFU/g among positive specimens in the dropped and control groups was 65 (8–150) and 10 (2–60), respectively ($P = 0.0003$). No patient developed postoperative SSI.
Conclusion Intraoperative hamstring autograft contamination rates were high. Hence, routine prophylactic decontamination of all hamstring autografts after harvesting and preparation and before implantation is recommended.
Level of evidence Controlled laboratory study.

Keywords ACL · Contamination · Hamstring · Dropped · Infection

Introduction

Infection is a rare but devastating complication following ACL reconstruction. In the recent literature, the reported incidence rates for septic arthritis after ACL reconstruction ranged from 0.14 to 1.8% [4, 5, 14, 16, 29, 33, 36].

Certain graft types have been linked to a higher risk of surgical site infection (SSI). Previous studies have shown higher overall rates of infection after ACL reconstruction with hamstring autograft compared with allografts or BPTB autografts [4, 7, 16, 19, 23, 36]. The underlying mechanisms contributing to graft-based differences in infection risks are unclear. However, several processes may contribute to the increased risk of infection associated with the use of hamstring autografts for ACL reconstruction, like excessive dissection with the potential of haematoma formation and the proximity of the surgical wound for harvesting site to the tibial tunnel [11, 22]. In addition, the relatively superficial position of the graft fixation and presence of suture materials, especially with the relative shortness of hamstring autografts, could create vulnerability to bacterial colonization and contamination of the joint [5, 16, 19, 22]. Finally,

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hamstring graft contamination during harvesting and preparation could be a possible source of SSI [30].

Previous studies were limited in that they did not quantitatively evaluate the level of contamination in contaminated ACL grafts [1, 2, 13, 25, 36, 37]. Additionally, data comparing graft contamination during harvesting and preparation with that of dropped specimens are limited [2, 28].

The primary aim of the present study was to estimate the risk of graft contamination by calculating contamination rates and determining the bacterial type in prospectively sampled hamstring grafts used in ACL reconstruction. Patients with positive cultures were clinically monitored for any signs of infection. Furthermore, the contamination of autograft tissue dropped on the OR floor was evaluated.

An additional aim of this study was to address the significance of contamination encountered after harvesting and preparation and upon dropping graft specimens on the floor, in order to provide information on the need for routine prophylactic decontamination of hamstring autografts after harvesting and preparation and before implantation, as well as the safety of saving of reusing a contaminated autograft after being accidentally dropped in the OR. This objective was achieved by quantitatively analysing the contamination level, measured in colony-forming units per gram of tissue (CFU/g), in consideration of the 10^5 CFU/g threshold for infection reported in the literature [6, 21].

The hypothesis for the present study was that the expected intraoperative contamination rate of soft tissue autografts is high with a low contamination level below the 10^5 CFU/g infection threshold.

Materials and methods

A total of 100 fresh ACL hamstring autograft specimens (semitendinosus and gracilis tendons) were retrieved from 50 primary ACL reconstruction surgeries. The procedures were performed by a single surgeon, and sample preparation and collection were performed by a single investigator (orthopaedic resident). All patients received prophylactic antibiotics (intravenous cefazolin [1–2 g] within 30 min preoperatively). In each case, sterile adhesive drapes were applied to cover the entirety of exposed skin at the surgical side. A 3–4-cm vertical skin incision was made directly over the tendons. Then, the sartorius fascia was opened, and the semitendinosus and gracilis tendons were both identified and harvested by an open-ended tendon stripper.

One specimen was obtained from the graft immediately after harvesting and dropped onto the OR floor adjacent to the operating table (dropped group); no changes were requested regarding the OR cleaning protocol. Each dropped specimen was left on the floor for 5 s before being collected by an assistant wearing sterilized gloves using sterilized forceps and placed in a sterile container with no decontamination.

Then, an assistant carried out the graft preparation at a side table. Non-absorbable sutures (fibreWire) were used during preparation. The second specimen (control group) was obtained at the end of the procedure, after graft implantation, from the excess graft that extended out from the tibial tunnel, just prior to graft fixation by bioabsorbable interference screw. All samples from both groups were placed in sterile containers using sterile forceps to prevent contamination. Each retrieved specimen was collected and immediately transported by the independent investigator to the microbiology laboratory for tissue culture in empty sterile containers. Each tissue sample was weighed using a digital balance and then rolled onto a sheep blood agar (SBA) plate and MacConkey (MAC) plate for at least 20 s to ensure that all sides of the specimen came in contact with the culture media; thereafter, the tissue was transferred to Robertson cooked meat liquid medium (RCM). The SBA and MAC plates and the RCM tubes were incubated in 5% CO_2 for 72 h to allow for aerobic growth and for 7 days to allow for fastidious and anaerobic bacterial growth. Colonial morphology and Gram stain assessments were performed for all the isolated organisms using standard microbiological methods. Microorganisms were identified using an automated identification system (MicroScan WalkAway-96 System; Dade Behring, West Sacramento, CA, USA) with identification and susceptibility panels (Negative Combo 42 and Positive Combo 28). Colony-forming units per gram were calculated from the total number of CFUs per plate using a colony counter (Schuett Biotec.de Count D-37079, Gottingen, Germany).

All patients underwent routine clinical follow-up, and any signs of SSI such as wound complications, fever, septic arthritis, recurrent effusion, failed surgery, or requiring a revision operation were recorded.

The institutional review board of King Saud University granted approval for the study before it was conducted (No.: 13/3824/IRB).

Statistical analysis

Statistical analysis was performed using SPSS software version 21 (IBM Corp., Armonk, NY). Data for dichotomous variables are expressed as percentages and were compared by using the χ^2 test. Data for the quantitative variable, CFU/g, are expressed as median and range. The Kolmogorov–Smirnov test of normality indicated that the CFU/g data did not follow a normal distribution; therefore, the nonparametric Mann–Whitney test was used to compare CFU/g between the control and dropped groups. *P* values less than 0.05 were considered statistically significant.

Sample size estimation was performed before conducting the study. It was calculated on the basis of the most recently

reported contamination rates among hamstring autografts after harvesting (16.7%) and when dropped on the floor (50%) [2]. The calculations, with an alpha cut-off of 5% and the beta being set at 20%, indicated the need to collect at least 30 specimens in each group to reach 80% power.

Results

The number of positive cultures for the control and dropped groups was 11/50 (22%) and 16/50 (32%), respectively. The difference in the contamination rate between groups was not statistically significant (n.s.).

The median (range) CFU/g for dropped and control specimens with positive cultures was 65 (8–150) and 10 (2–60), respectively. Specimens with positive cultures in the dropped group had a statistically significantly higher CFU/g than those in the control group ($P = 0.0003$).

The most common organisms identified in the control group were *Staphylococcus epidermidis* (45.5%) followed by *S. aureus* (36.4%).

In the dropped group, the most common organisms were *S. epidermidis* (31.3%) followed by *Bacillus* species (25%).

The median (range) clinical follow-up was 15 months (12–18 months). No patient developed any signs of SSI or required any further procedures.

Discussion

The most important finding of the present study is that a high rate of contamination can be expected during the harvesting and preparation of hamstring autografts.

The reported contamination rates of ACL autografts range from 2 to 23% [2, 12, 13, 24, 28]. In the current study, the relatively high contamination rate during harvesting and preparation (22%) could be explained by our retrieval of specimens from the graft at the end of the procedure just prior to graft fixation. This collection time was intentionally chosen to capture the potential risk of contamination when leaving the graft on a preparation table. The actual rate of contamination is likely to be underestimated if the specimen is retrieved immediately after harvesting, as shown by Hantes et al. [13] who found that the contamination rate increased during the waiting period between preparation and implantation. In our study, all samples were retrieved before graft fixation to eliminate potential sources of contamination via the instruments used for graft fixation [12, 16].

Bacterial contamination could also occur at the time of graft implantation, especially if the graft contacts the skin. The bacteria identified in the study by Maletis et al. [19] were consistent with typical skin flora. During arthroscopic knee surgery, the skin, after initial aseptic preparation, remains uncovered throughout the operation, and its residual flora are exposed to the surgeon's gloves and surgical instruments and, through them, the graft [13]. Nevertheless, in our study, a high rate of contamination was observed despite using sterile adhesive drapes to cover the entirety of exposed skin at the surgical side.

Another possible explanation is that hamstring autografts are contaminated by the instruments used during harvesting. Failure to fully disassemble a "tube-within-a-tube" hamstring harvester before sterilization may lead to unsatisfactory sterilization, providing a potential source for contamination [34].

Intraoperative contamination of autografts can also occur by accidentally dropping the graft onto the OR floor, which is the most commonly reported cause of contamination [9, 15]. Despite the great care taken during ACL reconstruction procedures, unintentional drops of grafts on the OR floor have been reported by at least one in four orthopaedic surgeons [15]. The reported contamination rates of dropped ACL autograft specimens range from 23 to 50% [2, 3, 17, 28]. In our study, the contamination rate in the dropped group was 32%. A retrieval time of 5 s was chosen because no significant difference has been reported between the contamination rates with shorter (5 s) or longer retrieval times (15 s) [28]. Furthermore, the duration of 5 s reflected the actual time needed to pick up an accidentally dropped graft.

To estimate the actual rate, level, and type of contamination related to being dropped, graft specimens in the dropped group were obtained immediately after harvesting and before preparation to avoid any further risk of contamination. However, graft contamination during harvesting and before dropping the specimen on the floor remains a potential source for overlapping contamination.

The risk of developing SSI after microbial contamination of the surgical site depends on the level of contamination, measured by CFU, virulence of the pathogen, and patient immunity [18, 20, 21]. The risk of surgical wound infection is considered to be elevated when the level of contamination exceeds 10^5 CFU/g of tissue [18]. Nevertheless, a dose as low as 100 CFU of staphylococci/g can lead to an infection in the presence of foreign bodies such as sutures [20]. In our study, the level of contamination of autografts after harvesting and preparation or after dropping on the OR floor was significantly lower than this threshold (10^5 CFU/g) [18]. However, with the use of hamstring autografts, preparation typically requires using sutures and possibly screws for fixation, which increase the risk of infection even with a low contamination level ($<10^5$ CFU/g). Therefore, the contamination level observed in the present study, while low, should be considered significant.

The virulence of organisms varies in terms of the tissue damage caused by their produced toxins. *Staphylococcus aureus* and coagulase-negative staphylococci were the most

commonly identified organisms in our study, consistent with previously reported organisms isolated from contaminated grafts during harvesting and preparation or from septic arthritis [12, 13, 24]. Coagulase-negative Staphylococcus and Bacillus species had low pathogenicity and have been reported as the most common contaminants of the OR floors [32]. *Staphylococcus aureus*, isolated from 4 of 11 contaminated grafts in the control group, was found to have a very low CFU/g, which may explain why no infections were encountered despite the high pathogenicity of the organism.

Limitations of the present study include not obtaining a swab culture from the harvester or sutures that were used during graft preparation, as they can be potential sources for contamination. Additionally, the incubation period for microorganism culture was 7 days, which might not be sufficiently long for slow-growing microorganisms such as *Propionibacterium acnes*, *Peptostreptococcus*, and *Corynebacterium* species, which require prolonged (14-day) incubation periods [8, 31].

In the series for this study, there was no association between graft contamination and postoperative infection since there were no clinical infections reported. The assessment was based on the presence of clinical signs and symptoms of SSI during follow-up, with no measurement of the erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) levels. Furthermore, subclinical infection signs such as prolonged pain, stiffness, and persistent effusion may have been overlooked. Additionally, this cohort is too small to conclude that the prevalence of infection is low in patients with positive culture.

Despite the low contamination level of hamstring autografts following harvesting and preparation, the presence of foreign bodies such as sutures increases the risk of infection with lower levels (CFU/g) of microorganisms. Thus, routine prophylactic decontamination of all hamstring autografts after preparation and before implantation is advised. Furthermore, presoaking the graft in gauze saturated with vancomycin was shown to be effective in reducing the infection rate following ACL reconstruction [10, 26, 27, 35]. Because of the low level of contamination and low pathogenicity of organisms identified in dropped specimens, saving and reusing hamstring autografts after proper decontamination is recommended over discarding them.

Conclusion

Although the intraoperative hamstring autograft contamination rates during harvesting and preparation or by accidentally dropping the graft onto the OR were relatively high, contaminated specimens demonstrated low bacterial counts, below the threshold for infection (10^5 CFU/g).

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Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interests.

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