Management of the Contaminated Anterior Cruciate Ligament Graft


Purpose: This systematic review explores management strategies for intraoperative anterior cruciate ligament (ACL) graft contamination. Methods: Two databases (Medline and EMBASE) were screened for studies involving ACL graft contamination published between 1946 and April 2013. We included studies evaluating the management of a contaminated graft and excluded small case-series studies. We conducted a full-text review of eligible studies, and the references were searched for additional eligible studies. Inclusion and exclusion criteria were applied to the searched studies. Results: Our search yielded 6 laboratory investigations with a total of 495 graft samples used. These samples were contaminated and cleansed by various methods. The most successful sterilization protocols used chlorhexidine or mechanical agitation with a polymyxin B—bacitracin solution to achieve sterility in 100% of their respective experimental graft tissues. A chlorhexidine soak and plain bacitracin soak were also effective, at 97.5% and 97%, respectively. Povidone-iodine and an antibiotic soak of polymyxin-bacitracin were the least effective, with sterility rates of 48% and 57%, respectively. Conclusions: The results of this review suggest that the optimal agent for sterilizing a dropped graft is chlorhexidine. A protocol of mechanical agitation and serial dilution with a polymyxin B—bacitracin solution was also highly effective; however, the sample size was too small to realistically recommend its use. Bacitracin alone was also found to be an effective sterilization agent, as was a combined solution of neomycin and polymyxin B. Pooled results showed that normal saline solution, povidone-iodine, and a polymyxin B—bacitracin solution all yielded suboptimal sterilization. The available evidence, however, is laboratory based and may not accurately reflect clinical conditions; moreover, there is a lack of biomechanical studies evaluating sterilized grafts. As a result, the findings should be interpreted with caution. Level of Evidence: Level IV, systematic review of basic science studies.
graft would appear to be the preferred choice if the tissue can be appropriately sterilized.\textsuperscript{7} This approach eliminates donor-site morbidity of a second harvest and obviates the use of an allograft, which is known to have higher failure rates, particularly in young active patients.\textsuperscript{8,9} Several small studies have been carried out to test the efficacy of various disinfection techniques; however, no consensus exists on optimal management after intraoperative ACL contamination.\textsuperscript{10-15}

The objective of this review was to determine the most effective method and agent to disinfect a contaminated ACL graft to provide evidence-based recommendations for surgeons faced with this complication.

**Methods**

**Identification of Studies**

Two reviewers (M.K. and F.M.) searched the Medline and Embase databases for studies involving the sterilization of soft-tissue grafts used for ACL reconstruction after intraoperative contamination. The search strategy was librarian assisted and combined the following terms: ACL, anterior cruciate ligament, contamination, graft contamination, microbial contamination, and drop (Table 1). Articles published between 1974 and April 2013 from Embase and between 1946 and April 2013 from Medline were included in this review.

The reviewers also completed a search of the references of recent reviews and each included article. The titles, abstracts, and full texts were screened for eligibility. The reviewers independently reviewed all studies, and any disagreement regarding data and study inclusion was resolved by discussion and consensus involving the senior author (O.R.A.).

**Assessment of Study Eligibility**

Studies meeting the following inclusion criteria were included in the review: (1) contained a discussion of a dropped or contaminated graft, (2) evaluated the management of a dropped or contaminated graft, and (3) published in English. The exclusion criteria were as follows: (1) non-experimental studies, (2) case studies with fewer than 5 patients (3) animal studies, and (4) review articles (except to review references).

**Data Abstraction**

Two separate reviewers collected the following data in an electronic spreadsheet (Microsoft Excel 2011; Microsoft, Redmond, WA): cleaning agent used, cleaning method, source of sample, number of samples, study design, study objectives, organisms used for inoculation, and outcomes measured. A quality assessment of all clinical studies was planned a priori using the Methodologic Index for Non-Randomized Studies (MINORS) scale developed and validated by Slim et al.\textsuperscript{16} in 2003. Given the ethical implications, we did not expect to obtain relevant randomized controlled trials meeting the eligibility criteria.

**Quality Assessment**

To our knowledge, no methodologic indices for the evaluation of in vitro studies have been developed or validated. Because only a few case series examining the rates of infection after sterilization of contaminated ACL grafts have been published, laboratory-based studies exploring the efficacy of various cleansing techniques may be used judiciously to inform clinical practice. To assess the methodologic quality of the included studies, an adapted MINORS scale\textsuperscript{16} for in vitro experiments was developed (Appendix Table 1). Two reviewers (M.K. and B.B.R.) independently conducted a quality assessment of the included articles.

Like the original MINORS scale, the adapted scale consists of 12 items, with 2 additional items proposed for future in vitro studies. Each item is scored from 0 to 2; for most items, 0 indicates that the item is not reported, 1 indicates that the item is reported but inadequately, and 2 indicates that the item is sufficiently reported (Appendix Table 1). The intraclass correlation coefficient was used to evaluate interobserver agreement for the continuous methodologic quality scores.

**Data Analysis**

Data abstracted from all included studies were organized into a table (Microsoft Excel 2011). Descriptive statistics were calculated to reflect the frequency and percentage of each outcome measure.

The $k$ statistic was used to examine the extent of agreement between the reviewers determining study eligibility. On the basis of the guidelines of Landis and Koch,\textsuperscript{17} a $k$ of 0 to 0.2 represents slight agreement; 0.21 to 0.40, fair agreement; 0.41 to 0.60, moderate agreement; and 0.61 to 0.80, substantial agreement. A value above 0.80 is considered almost perfect agreement.

We graded all studies for the level of evidence according to the criteria of Wright and Swiontkowski.\textsuperscript{18} They ranked the quality of evidence based on the
strength of research methodology, thus allowing for weighting and assessment of published research studies.

**Results**

**Identification of Studies**
Our initial search yielded 336 studies, of which 7 met the inclusion criteria10-15,19 (Fig 1). One study was not available despite contacting the authors,19 resulting in 6 studies being included in this review. The κ statistic was 0.84 (95% CI, 0.73 to 0.97) for the title and abstract review, indicating almost perfect agreement.

**Study Characteristics**
All included studies were laboratory investigations conducted between 1991 and 2012. There were a total of 495 samples used across all arms (including control groups). Three hundred twenty-eight samples were part of the various experimental groups. Samples included those from fresh-frozen human Achilles tendon–calcaneus grafts (15), fresh-frozen human patellar tendon–bone grafts (30), excess hamstring graft from ACL reconstructions (90), live human ACLs (150), human cadaveric ACLs (10), and human cadaveric patellar tendons (33). The inter-rater reliability between the reviewers for the 6 continuous quality scores was high (intraclass correlation coefficient, 0.97; 95% confidence interval, 0.82 to 0.99). The mean study score was 18 of 24 (range, 13.5 to 22). Study characteristics are listed in Table 2.

The samples were inoculated or contaminated using various procedures, from dropping the tissue samples onto an operating room floor to directly contaminating the...
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Location</th>
<th>Design</th>
<th>No. of Samples</th>
<th>No. of Samples in Trial Arms</th>
<th>Source</th>
<th>Inoculation Method</th>
<th>Cleaning Agent</th>
<th>Cleaning Method</th>
<th>Outcome</th>
<th>Mean MIIVS Quality Score</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burd et al.</td>
<td>2000</td>
<td>United States</td>
<td>Controlled trial</td>
<td>22</td>
<td>15</td>
<td>Human Achilles tendon–calcaneus</td>
<td>Polymicrobial inoculum pipetted onto tissue sample, 1 min</td>
<td>2% chlorhexidine + NS solution</td>
<td>2-3 min in NS, 7-8 min of irrigation</td>
<td>Culture</td>
<td>20/24</td>
<td>Chlorhexidine gluconate successful</td>
</tr>
<tr>
<td>Parker and Maschke</td>
<td>2008</td>
<td>United States</td>
<td>Controlled trial</td>
<td>40</td>
<td>30</td>
<td>Human bone–patellar tendon–bone</td>
<td>Determined bacterial flora on OR floor and then created highly concentrated representative suspension</td>
<td>Polymyxin–bacitracin, pulsatile lavage + abx soak, mechanical agitation, and serial dilution + abx soak</td>
<td>Mechanical agitation and serial dilution (10 × 15-s shakes), abx soak, or pulsatile lavage</td>
<td>Semiquantitative culture</td>
<td>21/24</td>
<td>Mechanical agitation and serial dilution comprise reliable technique</td>
</tr>
<tr>
<td>Stanford et al.</td>
<td>1999</td>
<td>Australia</td>
<td>Controlled trial</td>
<td>33</td>
<td>33</td>
<td>Human cadaveric patella–patellar tendon</td>
<td>Grew reference strains (1 × 10⁶/mL) and then submerged graft for 5 min</td>
<td>10% povidone–iodine at room temperature/36°C or NS</td>
<td>Static soaking (2 × 5 min) or serial washing with agitation (5 min, 3 Hz, 3-cm amplitude × 5)</td>
<td>Turbid appearance of culture solution</td>
<td>22/24</td>
<td>10% povidone-iodine was not effective</td>
</tr>
<tr>
<td>Molina et al.</td>
<td>2000</td>
<td>United States</td>
<td>Controlled trial</td>
<td>200</td>
<td>150</td>
<td>Human ACL from patients undergoing knee arthroplasty</td>
<td>Dropped on OR floor, 15 s</td>
<td>Neomycin—polymyxin B, 10% povidone-iodine solution, and standard chlorhexidine gluconate solution</td>
<td>Soak for 90 s</td>
<td>Culture</td>
<td>15.5/24</td>
<td>Dropped graft does not always imply contamination; chlorhexidine produced lowest culture results</td>
</tr>
<tr>
<td>Plante et al.</td>
<td>2013</td>
<td>United States</td>
<td>Controlled trial</td>
<td>180</td>
<td>90</td>
<td>Excess hamstring tendon from ACL reconstructions</td>
<td>Dropped on OR floor, 5 s or 15 s</td>
<td>Saline solution, bacitracin, or 4% chlorhexidine</td>
<td>Rinse for 3 min</td>
<td>Culture</td>
<td>13.5/24</td>
<td>S aureus most common isolate, 4% chlorhexidine and bacitracin equally effective</td>
</tr>
<tr>
<td>Cooper et al.</td>
<td>1991</td>
<td>United States</td>
<td>Controlled trial</td>
<td>20</td>
<td>10</td>
<td>Human cadaver ACL</td>
<td>Dropped on OR floor, 3 min</td>
<td>Bacitracin—polymyxin B + NS rinse</td>
<td>Soak for 15 min and then saline solution rinse × 3</td>
<td>Culture</td>
<td>16/24</td>
<td>15-min soak was not sufficient</td>
</tr>
</tbody>
</table>

abx, antibiotic; MIIVS, Methodological Index for In Vitro Studies; NS, normal saline solution; OR, operating room.
samples with common pathogens, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, Klebsiella pneumoniae, diphtheroids, and micrococcus species. They were then cleansed with a variety of cleaning solutions, including 2% and 4% chlorhexidine, normal saline solution, antibiotic solutions of polymyxin B and bacitracin or polymyxin B and neomycin, and 10% povidone-iodine. The cleaning methods included 7 to 8 minutes of irrigation, mechanical agitation, serial dilution, pulsatile lavage, soaking for a variable duration of time, or a rinse and soak/rinse combination (Table 3).

The culture techniques used to evaluate the presence of contamination in the samples are outlined in Table 4. None of the studies were able to assess for clinical evidence of infection because none of the graft samples were used in an ACL reconstruction after being used in the experiment.

### Outcomes
The most successful sterilization protocols were 7 to 8 minutes of irrigation with 3 L of 2% chlorhexidine and mechanical agitation and serial dilution with a polymyxin B–bacitracin solution, which both

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Samples in Experimental Arms (Total No. of Samples)</th>
<th>Contaminant</th>
<th>Cleaning Agent</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burd et al.12 (2000)*</td>
<td>15 (22)</td>
<td>*S. aureus, S. epidermidis, P. aeruginosa, K. pneumoniae</td>
<td>Pulsatile irrigation (7-8 min) with 3 L of 2% chlorhexidine (10)</td>
<td>10/10 sterile (*P = .0001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pulsatile irrigation (7-8 min) with 3 L of NS (5)</td>
<td>0/5 sterile</td>
</tr>
<tr>
<td>Parker and Maschke14</td>
<td>30 (40)</td>
<td><em>S. aureus, P. aeruginosa,</em> Bacillus, diphtheroids</td>
<td>Antibiotic (100 mL) soak (polymyxin B [166.66 U/mL] and bacitracin [16.66 U/mL])</td>
<td>0/10 sterile; mean, 17.4 CFU (*P = .5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pulsatile irrigation</td>
<td>6/10 sterile; mean, 0.6 CFU (*P = .007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mechanical agitation and serial dilution with 100 mL of antibiotic polymyxin B and bacitracin solution (×10)</td>
<td>10/10 sterile (*P = .008 against control and *P = .02 against pulsatile lavage)</td>
</tr>
<tr>
<td>Stanford et al.15</td>
<td>33 (36)</td>
<td><em>S. aureus</em></td>
<td>Static soak with 10% povidone-iodine</td>
<td>0/6 sterile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Room temperature 36°C</td>
<td>1/6 sterile</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>P. aeruginosa</em></td>
<td>Static soak with 10% povidone-iodine</td>
<td>0/6 sterile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Room temperature 36°C</td>
<td>1/6 sterile</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
<td>Serial washing with 10% povidone-iodine and agitation (5 min at 3 cycles/s with 3-cm amplitude × 5)</td>
<td>1/9 sterile</td>
</tr>
<tr>
<td>Molina et al.10 (2000)</td>
<td>150 (200)</td>
<td>OR floor after knee arthroplasty, 15 s</td>
<td>1 mL of 40 mg of neomycin sulfate and 200,000 U of polymyxin B sulfate in 1 L of NS, 90 s</td>
<td>47/50 sterile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10% povidone-iodine, 90 s</td>
<td>38/50 sterile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4% chlorhexidine, 90 s</td>
<td>49/50 sterile</td>
</tr>
<tr>
<td>Plante et al.11 (2013)</td>
<td>90 (180)</td>
<td>OR floor during ACL reconstruction, 15 s</td>
<td>NS soak, 3 min</td>
<td>21/30 sterile</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4% chlorhexidine soak, 3 min</td>
<td>29/30 sterile (*P = .03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Soak with bacitracin, 50,000 U/L; 3 min</td>
<td>29/30 sterile (*P = .03)</td>
</tr>
<tr>
<td>Cooper et al.13 (1991)</td>
<td>10 (20)</td>
<td>OR floor after hip arthroplasty, 3 min</td>
<td>Soak with 50,000 U of bacitracin and 500,000 U of polymyxin B in 1.5 L of NS, 15 min, and then sterile NS rinse × 3</td>
<td>7/10 sterile (*P = .37)</td>
</tr>
</tbody>
</table>

CFU, colony-forming units; NS, normal saline solution; OR, operating room.

*Only part 3 of the study was included.
showed sterility in 100% of their respective experimental graft tissues (10 of 10 samples). Soaking the contaminated graft in 4% chlorhexidine for 90 seconds showed sterility rates of 98% (49 of 50 samples) and 96% (29 of 30 samples) in 2 studies. Overall, the use of chlorhexidine with any method resulted in a successful sterilization rate of 98% (88 of 90 samples). A combined solution of neomycin and polymyxin B was successful in 94% of cases (47 of 50 samples). A normal saline solution soak resulted in a sterilization rate of only 70% (21 of 30 samples). When pulsatile lavage with normal saline solution was used, data pooled from 2 studies showed a sterilization rate of 40% (6 of 15 samples). Altogether, the use of normal saline solution overall resulted in a sterilization rate of 60% (27 of 45 samples). Bacitracin mixed with polymyxin B resulted in a sterilization rate of 57% (17 of 30 samples); however, bacitracin by itself was found to have a higher sterilization rate of 97% (29 of 30 samples). Povidone-iodine resulted in successful sterilization in only 48% of cases (40 of 83 samples) from 2 studies, although the data were highly heterogeneous between these studies. Molina et al. found povidone-iodine to be successful in 76% of cases (38 of 50 samples), whereas Stanford et al. found it to be successful overall in 11% of cases (3 of 27 samples) (Table 3).

**Discussion**

The risk of contamination after a graft has been dropped is high: 60% of contaminated dropped tissue grafts yield positive bacterial cultures after 10 days. Contamination can also occur without dropping the graft as shown in a Level II study in which 12% of ACL autografts were contaminated during preparation for the reconstruction; however, there was no clinical evidence of infection. In the case of a dropped graft, the correct protocol and sterilization agent necessary for decontamination should be readily available if graft retention is considered. Sterilization and retention of the graft result in the least morbidity to a patient and comprise the most appealing option for a contaminated graft, provided an optimal protocol is identified.

A survey of sports medicine specialists raised the question of intraoperative graft contamination management when performing ACL reconstruction. Among surgeons, it reported that 49 of 196 respondents (25%) had a combined 57 contaminated grafts, 43 of which (75%) were sterilized and used in the planned procedure; the remainder were discarded and substituted with either a different autograft or an allograft was used. Among those who elected to disinfect the graft, the disinfecting solution was variable, but the majority chose chlorhexidine. Among those who chose chlorhexidine, the method and time of delivery varied. Most surgeons soaked the graft in chlorhexidine solution for periods ranging between 90 seconds and 30 minutes. Supplemental methods reported also included pulsatile lavage or mechanical agitation of tissues. Among surgeons who had not had a graft contamination, 58% of respondents hypothetically stated that they would cleanse the graft and continue with the procedure. Furthermore, there were no reported clinical infections in any of the cases.

Our review of the literature identified 6 laboratory studies evaluating various sterilization solutions and methods to treat a contaminated graft. Given the heterogeneity of the protocols evaluated, we are unable to recommend an ideal protocol. However, key findings from this review indicate that chlorhexidine sterilization was found to be most effective. Of 90 contaminated samples that were sterilized with various methods of chlorhexidine, 98% were found to be successfully treated. Other highly effective sterilization options were the use of bacitracin alone and the use of neomycin and polymyxin B in a combined solution, although these results were from 1 study each and had smaller sample sizes.
sizes in comparison to the pooled chlorhexidine samples. One study showed 100% success with mechanical agitation and serial dilution with 100 mL of an antibiotic polymyxin B and bacitracin solution; however, the sample size was limited to 10 cases. It is interesting to note that bacitracin combined with polymyxin B yielded significantly poorer findings in comparison with bacitracin alone. Normal saline solution and povidone-iodine were found to be least effective across the studies.

Few case series exist in the literature on the subject of this review. In a published case series of 3 contaminated ACL grafts sterilized and used in reconstruction, Pasque and Geib identified situational awareness of all members of the surgical team as essential in preventing a dropped graft. Recommendations from their case series closely followed the results of Goebel et al., with immediate removal of the graft from the floor; removal of all suture material; and 15 to 30 minutes of cleansing of the graft with chlorhexidine and triple antibiotic solution, followed by a rinse. Oral and intravenous antibiotics were given for 1 to 2 weeks. No cases of septic arthritis were reported after treatment with this protocol.

The results of our systematic review indicated excellent outcomes (regarding bacterial growth) with the use of both 2% and 4% chlorhexidine solutions. Burd et al. used a bovine model and confirmed excellent sterilization outcomes with 2% and 4% chlorhexidine solutions, with lesser concentrations found to be less effective. However, it is also important to consider the effect of the sterilizing solutions on the mechanical properties of the grafts. Chlorhexidine belongs to the bisbiguanide class of antiseptics, and it has documented cytotoxicity against fibroblasts and negatively affects cell proliferation. However, its effect on wound healing is controversial.

There has been significant concern regarding the harmful effects of chlorhexidine on tissue and native joint integrity. A bovine tissue study suggested that 2% chlorhexidine is a safe agent to use on a tissue graft; however 4% chlorhexidine dissolved collagen fibrils even after short incubation times and therefore would alter the biomechanical properties of the graft. The effect of using power irrigation was explored by Han et al. in a bovine model. They reported that disinfecting tendons with 3 L of 2% chlorhexidine with power irrigation did not adversely weaken the tensile mechanical properties of the tendon. The sterilization procedure in this study was similar to that of Burd et al. Specifically, 8 fresh bovine superficial digital flexor tendons and contralateral tendons serving as controls were loaded to failure after sterilization. Given the limitations of a bovine model, it is reassuring to note that ligament biomechanical integrity was maintained with this sterilization protocol.

There are significant legal implications to consider with a contaminated graft. The surgeon must be prepared to use an alternative graft source in the case of contamination, and therefore obtaining informed consent from the patient regarding this potential complication would be prudent preoperatively. This is particularly the case because a contaminated graft may constitute a “never event,” or an inexcusable action in the health care setting, and a surgeon may be denied the option to implant a previously contaminated graft.

There are multiple strengths to this review. The literature search was extensive and included multiple reviewers during article screening, evaluation, and data abstraction to minimize selection bias, with excellent agreement for study inclusion. In addition, eligibility criteria were sufficiently broad, allowing for inclusion of more eligible studies related to human ACL graft sterilization techniques.

Given the varied techniques and sterilization solutions used in the literature, future research should focus on development of a database to collect clinical data, thereby allowing the identification of the optimal techniques of graft sterilization. It is neither feasible nor ethical to conduct a controlled trial given the rare occurrence of ACL graft contamination, as well as the severe consequences that a patient may experience. A large national or international registry would allow clinical data to be obtained and analyzed with clinical outcome measures. On the basis of the results of this review, future studies should focus on chlorhexidine and/or mechanical agitation and serial dilution with a polymyxin B and neomycin solution to determine the efficacy of sterilization and to assess the maintenance of tissue integrity.

**Limitations**

A limitation of our study is the use of varied and differing concentrations of cleaning agents or sterilization techniques, making it difficult to pool data findings. The bacterial contaminants also varied in the studies. In addition, basic science studies are hypothesis generating and do not provide a definitive recommendation for handling ACL contamination. All studies included in this review were laboratory evaluations because limited clinical data exist in the form of a few case studies. Data from these laboratory results may be extrapolated to clinical practice, but this should be done with caution. It is important to note that the proposed index for evaluating the methodologic quality of the included studies has not been validated with regard to content or scoring. Another limitation of the included studies was the absence of biomechanical analysis of the tensile strength of the tendon after the various sterilization protocols. Although data exist in the literature regarding bovine graft tensile strength with power irrigation and chlorhexidine solution, the relation...
between neomycin, polymyxin B, bacitracin, or povidone-iodine sterilization techniques and graft integrity has not been explored.

Conclusions

The results of this review suggest that the optimal agent for sterilizing a dropped graft is chlorhexidine. A protocol of mechanical agitation and serial dilution with a polymyxin B and bacitracin solution was also highly effective; however, the sample size was too small to realistically recommend its use. Bacitracin alone was also found to be an effective sterilization agent, as was a combined solution of neomycin and polymyxin B. Pooled results showed that normal saline solution, povidone-iodine, and a polymyxin B solution all yielded suboptimal sterilization. The available evidence, however, is laboratory based and may not accurately reflect clinical conditions; moreover, there is a lack of biomechanical studies evaluating sterilized grafts. As a result, the findings should be interpreted with caution.

References
## Methodological Index for In Vitro Studies*

1. Clearly stated purpose: The question addressed in the study is explicitly stated and testable by statistical means
2. Adequate control groups: Because graft contamination can occur without dropping, 2 control groups exist for in vitro studies
   - 0: control groups not adequately described
   - 1: control group of non-contaminated graft OR non-sterilized graft
   - 2: control groups of non-contaminated graft AND non-sterilized graft
3. Graft type: Description of graft material (tissue type, autograft/allograft, and devitalized/viable)
4. A priori power analysis: Justification of sample size for both experimental and control groups needed to determine statistical significance
5. Appropriate statistical analysis: Description and implementation of statistical tests appropriate to dataset with reported P values
6. Unbiased assessment of outcome: Objectivity of methodology/evaluator used to determine successful sterilization
   - 0: no description of outcome criteria AND evaluator
   - 1: qualitative/subjective methodology (turbidity, culture plates) with unblinded evaluator
   - 2: qualitative/subjective methodology with blinded evaluator (must be explicitly stated as blinded) OR quantitative methodology (RT-PCR, photospectrometric assays, automated counting)
7. Contaminant identity: The organism type and quantity used for inoculation are described
   - 0: not described
   - 1: organism type OR organism quantity (if graft dropped on floor, organism type may be determined by swab of operating room floor and reported in results)
   - 2: organism type AND organism quantity
8. Infection risk: Intraoperative graft contamination is most commonly due to dropping, but in vitro studies may expose grafts to inoculum concentrations exceeding those encountered in clinical scenario
   - 0: not described
   - 1: graft dropped on floor OR inoculated with flora at same concentration as that found on operating room floor (<10⁵ CFU/organism)
   - 2: graft inoculated with concentrated bacterial load that exceeds that found on operating room floor (≥10⁵ CFU/organism)
9. Culture method: Description of how contaminated grafts were cultured in sufficient detail to repeat (or detailed methodology is referenced)
10. Culture time: Because some infections may develop slowly, longer intervals of culture may be needed to ensure successful sterilization
    - 0: not reported
    - 1: culture of ≤6 d
    - 2: culture of ≥7 d
11. Cleansing agent: Description of agent used for sterilization, including identity, concentration, and volume
12. Cleansing method: Description of physical or mechanical conditions in which the graft was sterilized, including cleansing time
13. Biomechanical properties: Sterilized grafts are compared against control groups to determine any adverse effects of cleansing protocol on biomechanical properties (0, biomechanical testing not performed; 1, tensile testing [structural and mechanical properties] OR kinematic testing [graft function and forces]; or 2, tensile testing AND kinematic testing)
14. Cytocompatibility: Toxicity of sterilized grafts on co-cultured cells is investigated; this does not include resident cells of autografts (because comparison of cytotoxicity between resident cells of autografts and devitalized allografts is not possible)

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*Unless otherwise noted, the items are scored as follows: 0, not reported; 1, reported but not adequately; or 2, reported adequately.

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CFU, colony-forming units; RT-PCR, reverse transcriptase—polymerase chain reaction.