Reviewer’s report

Title: Which is the best method of sterilization for recycled bone autograft in limb salvage surgery: A radiological, biomechanical and histopathological study in rabbit.

Version: 3
Date: 19 October 2014
Reviewer: Steven Thorpe

Reviewer’s report:

Major Compulsory Revisions:

1) The authors should comment on the study design to only follow specimens out to 3 months. Previous literature has demonstrated an average time to union in human case series of 9.5 months (Sugiura et al Arch Orthop Trauma Surg 2012). Limiting the study period to only 3 months may restrict the ability to know the real outcome of the graft for each sterilization procedure. See comments below with regards to biomechanical analysis.

2) In all biomechanical tests (Figures 4 and 5), biomechanical performance of the graft decreased steadily after week 9 (especially the irradiated and pasteurized groups). The authors need to discuss this in the discussion section. Is the graft undergoing resorption, and thus losing its mechanical properties. What would the grafts look like at 6 months and at 1 year?

3) While a complication analysis was not mentioned in the aim of this study, there is no mention in results of any infections (superficial or deep). This should be noted in the paper, as infection is commonly noted in the recycled autograft literature with substantial frequency.

4) The authors report outcomes for histological analysis (Table 8) – but there is no statistical analysis of the data reported. The authors only note that the autoclaved specimens demonstrated the lowest points in all categories at the end of 12 weeks.

5) The authors chose 6 animals for each arm of the experimental analysis, resulting in a total of 24 animals. The authors should describe why they chose this number of specimens. The authors should clearly delineate how their power analysis was performed.

6) In the conclusion of the abstract, the authors’ first statement is that irradiation does not significantly alter the biomechanical properties when compared to untreated samples. This is contradictory to the conclusion in the paper.

Minor Essential Revisions:

1. In laying out the purpose or aim of the study the authors state that the purpose of the study is “to determine the most effective way to process the bone
autografts without compromising the bone healing and structural properties.” The use of “effective” may not be appropriate, as the definition of effective sterilization process would necessitate an analysis of tumor cell viability after sterilization, which was not part of this study.

2. Even though an independent pathologist performed the histologic analysis, a statement about validity and inter-observer reliability for the semi-quantitative system used for histologic analysis should be added.

3. A separate stats paragraph should be included in the methods section of this manuscript. Within this paragraph, the authors should describe the statistical analysis for each subsection of the analysis. The use of the ANOVA test with post hoc analysis is appropriate. The description of the p-value set for significance should be listed here. If power analysis was truly done, then it should be stated here.

4. Line 285: notes the rabbit (shown in Figure 2) has an “unusual reaction” to the autoclaved bone, but the authors note that the rabbit was doing well till end of study period. How with a “deformed, shortened left leg” and the radiographs shown, can that statement be true? Could the results for this specimen be second to infection? Was this analyzed? Were any infections observed?

5. First paragraph of the discussion section needs to summarize the key results and highlight their importance in terms of answering the primary question of the paper: which method of sterilization is the best.

6. Table 6 should be made more concise by making one table that reports number of sites of union per method of sterilization and control at the various time points.

7. Overall scientific writing should be improved to present the data in more concise format. For instance results can be noted as “significant (p = x)”.

8. The purpose statement of the study in the abstract (lines 54-56) should be moved to the introduction.

9. Table legends need to be more descriptive. For instance Table 6 should be titled as “Macroscopic analysis of union at proximal and distal graft sites.”

10. Figure legends need to be significantly reduced. Interpretation and discussion of the results should not be included in the Figure legend.

Discretionary Revisions

1) The authors could discuss any thought to the use of fibular autograft to augment the reimplantation of the recycled autograft (that has lost osteoinductive properties as part of the sterilization process).

2) It would be helpful to list known complication rates from various reconstruction methods in the literature. i.e. endoprosthesis, allograft, and recycled autograft.
3) It would be beneficial to see a Figure with representative photomicrographs of a histology specimen that was used to determine quantified numerical score per Table 7. Demonstrate how a numerical value was given for callus formation, % osteocytes, % bone marrow.

4) The authors may want to note why liquid nitrogen was not tested given that its use has been well documented in the literature.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.