**EFFECT OF CHEMICAL STERILIZATION ON THE MECHANICAL PROPERTIES OF HUMAN CORTICAL BONE ALLOGRAFTS**

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**Introduction**
Musculoskeletal allografts are increasingly being utilized in a variety of orthopedic procedures. However, they pose the serious risk of viral or bacterial disease transmission as highlighted by the 2001 death of a young man from *Clostridium sordellii* sepsis after receiving a contaminated allograft. Gamma irradiation has been widely used to minimize this risk, but has been shown to weaken musculoskeletal allografts. Therefore, the need exists for sterilization techniques that do not diminish allograft biomechanical properties. BioCleanse™ (Regeneration Technologies Inc., Alachua, FL) is a fully-automated, low-temperature, chemical sterilization process that removes endogenous donor material such as blood and lipids, and has been validated to consistently eliminate bacteria, bacterial spores, fungi, and viruses. The objective of this study was to evaluate the effects of this sterilization technique on bone strength.

**Methods**
Following Institutional Review Board approval, cortical bone specimens from 7 human cadaveric donors, ages 29-62, were machined into cylindrical geometries and randomly assigned to the following treatment groups:

1) **BioCleanse**: BioCleanse™ chemical sterilization
2) **BioCleanse + Irradiation**: BioCleanse™ and terminal sterilization by 20-23kGray of gamma irradiation while packed on dry ice to maintain water in solid phase
3) **Freeze Dried**: BioCleanse™, lyophilization, terminal sterilization by STERRAD (J&J, Inc.) and rehydration
4) **Control**: untreated

Axial compression (Figure 1), diametral compression (Figure 2), shear (Figure 3), and three-point bending tests (Figure 4) were performed to measure ultimate strength. Specimens for the axial (n=16 per group), diametral (n=25 per group), and shear (n=21 per group) test groups were obtained from the femur. For each testing method, the effect of treatment on the outcome was analyzed using generalized linear models. Contrast statements were generated to produce pair-wise comparisons among the four treatment groups. Adjusted p-values less than 0.05 were considered statistically significant.

**Results**
Axial Compression: The Freeze Dried group was significantly stronger than the BioCleanse, BioCleanse+Irradiation, and Control groups (p<0.05).

Diametral Compression: The Control group was significantly stronger than the BioCleanse+Irradiation and the Freeze Dried groups (p<0.05).

Shear: The Control group was significantly stronger than the BioCleanse+Irradiation and Freeze Dried groups (p<0.05).

Additionally, the BioCleanse group was significantly stronger than the BioCleanse+Irradiation group (p<0.05).

Bending: There were no significant differences among the four treatments.

**Table 1: Results. Similar superscript letters indicate p<0.05.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Axial Compression (MPa)</th>
<th>Diametral Compression (MPa)</th>
<th>Shear (MPa)</th>
<th>3-Point Bending (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>168.0 ± 17.1a</td>
<td>27.0 ± 3.0a</td>
<td>79.3 ± 7.9a</td>
<td>253.1 ± 26.3a</td>
</tr>
<tr>
<td>BioCleanse</td>
<td>165.5 ± 10.4d</td>
<td>25.4 ± 2.7b</td>
<td>78.4 ± 10.7b</td>
<td>246.2 ± 32.5b</td>
</tr>
<tr>
<td>BioCleanse + Irradiation</td>
<td>160.2 ± 13.9d</td>
<td>24.6 ± 2.5b</td>
<td>73.1 ± 6.7d</td>
<td>230.9 ± 38.2b</td>
</tr>
<tr>
<td>Freeze Dried</td>
<td>226.5 ± 44.6b</td>
<td>24.0 ± 3.2b</td>
<td>73.9 ± 13.1b</td>
<td>255.7 ± 38.7b</td>
</tr>
</tbody>
</table>

**Discussion**
The mechanical properties of human cortical bone allografts treated with the BioCleanse™ sterilization process are not significantly different from the untreated control. Although the decrease in ultimate diametral and shear strength from terminal sterilization with low dose gamma irradiation was statistically significant, the clinical significance may be minimal. Lyophilization and terminal sterilization by STERRAD appears to increase ultimate compression strength, but it also reduces ultimate diametral and ultimate shear strength. However, the rehydration time for lyophilized specimens will affect the material properties and likely is the main reason for the significant increase in axial compression strength. The clinical significance of these findings is that the BioCleanse™ chemical sterilization process has been shown to have no significant adverse effects on the biomechanical properties of cortical bone allografts.

**References**