



Assessment of the Antigenic Response of Sheep Recipients Following Implantation of BioCleanse® Treated Bovine Bone, Untreated Bovine Bone and Untreated Allograft Bone

Introduction

As an addendum to a study designed to analyze the comparative rate of biological incorporation of various implants in a sheep model through histopathological and biomechanical evaluation, this study was performed to assess the antigenicity of traditionally processed tissue in comparison to that processed by the BioCleanse® process. Specifically, this study evaluated the antigenic response elicited by bovine bone treated by 1) aseptic processing (Trad), 2) the BioCleanse® Tissue Sterilization Process (Bc), or 3) the BioCleanse® Tissue Sterilization Process followed by gamma irradiation (BcG) when implanted in a discordant recipient (sheep).

Methods

Twenty-four skeletally mature sheep each underwent staged bilateral surgical procedures. Two prepared 5 x 25mm tunnels in the medial proximal tibia received the test article bone dowels. Six sheep were implanted with untreated aseptic bovine xenograft in one limb and twelve weeks later received untreated aseptic bovine xenograft in the contra lateral limb (Group 1). A second group of six sheep were implanted with BioCleanse®-treated bovine xenografts in the same manner as the first group (Group 2). A third group of six sheep received untreated but aseptically handled sheep bone allografts (Group 3). A fourth group of six sheep received BioCleanse®-treated bovine xenograft sterilized with gamma irradiation in the same manner as the previous groups (Group 4). All of the animals were sacrificed 24 weeks following the initial surgery. Explant sites were harvested; bone was processed and embedded in plastic. 100µm thick sections were stained with Giemsa. Slides were analyzed on a numerical scale to evaluate inflammation and bone growth.

Heparanized blood was collected from representative sheep in each group, prior to implantation and 1, 3, and 6 months after implantation. Peripheral Blood Mononuclear Cells (PBMC's) were isolated from whole blood and suspended in media. Powder from each of the bone implants was mixed together into a separate suspension. Cell suspension and bone powder suspension of matching study groups were combined and incubated. Cell proliferation as determined by ³H-thymidine or MTT uptake was then measured at three days. A Stimulation Index was calculated as the fold increase over a media control for the PBMC's from each animal.

Results

The histopathological results at both 12 weeks and 24 weeks indicate that the BioCleanse®-treated bovine bone remodels comparably to sheep allograft bone. As shown in Figure 1, at 12 weeks, the most active response was in Group 1 (untreated bovine xenograft) where the implant was being actively removed by large numbers of osteoclasts accompanied by mixed inflammatory cells and fibrosis. Bone ingrowth, at the 12 week time point, was greatest in the BioCleanse®-treated bovine xenograft group (Group 2) and least in the untreated bovine xenograft group (Group 1). The greatest overall reactivity was in Group 1 tissues. Examples of histology slides for Group 1 and Group 2 at 12 weeks are shown in Figure 2. Both of the BioCleanse®-treated groups were found to be similar pathologically to the allograft control, eliciting healing responses similar to that of fresh frozen allograft when implanted into the proximal tibia of sheep.

Figure 3 summarizes the memory response of the various groups when challenged with the implant material at time points indicated in the X-axis as determined by the PBMC assay. The legend for the graph depicts respective groups of recipients and the implant material used to measure the recall response. The data is presented as the average stimulation index (fold increase in response to challenge material

over media control) for each group of recipients. Sheep receiving the traditionally processed bovine bone implants (Trad) demonstrated the highest recall response at 1-month post surgery. This response was significant ($p=0.008$, t-test) at 1 month post-surgery, and tapered to pre-bleed/background levels at 3 months.

Amongst the other groups, the BioCleanse[®]-implant recipient group (Bc) showed a slight, but not significant response to the implant material ($p>0.05$), and the allograft (Sheep), and gamma-irradiated BioCleanse[®] (BcG) implant recipients failed to demonstrate any measurable recall response to their respective implants. In all 4 treatment groups, the recall response reached background/pre-bleed levels by 3 months post-implant.

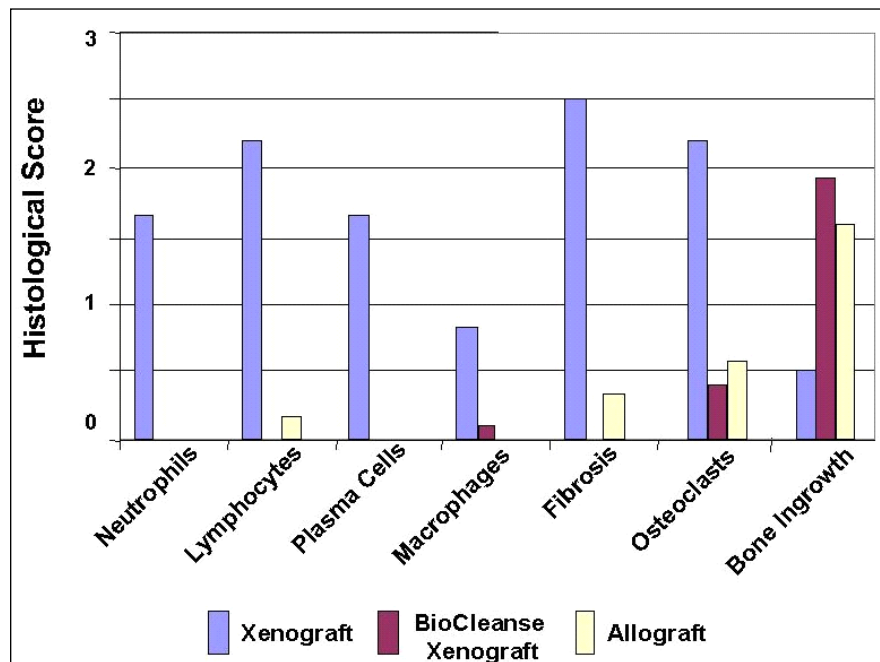


Figure 1: Histopathological comparison of untreated bovine xenograft, bovine xenograft processed through the BioCleanse[®] Tissue Sterilization Process, and untreated sheep allograft

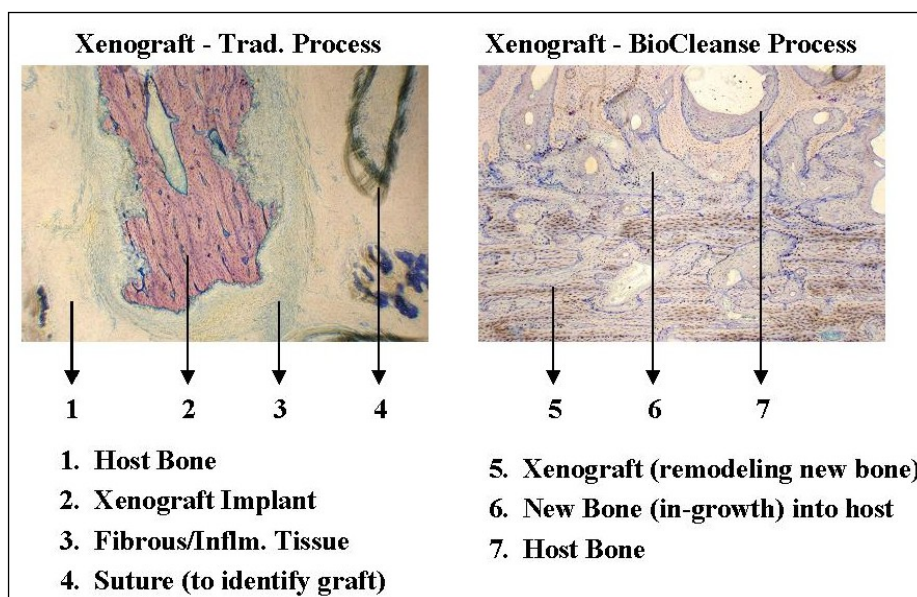


Figure 2: Example histology slides for the untreated bovine xenograft group (left) and the BioCleanse[®]-treated bovine xenograft group (right) at 12 weeks

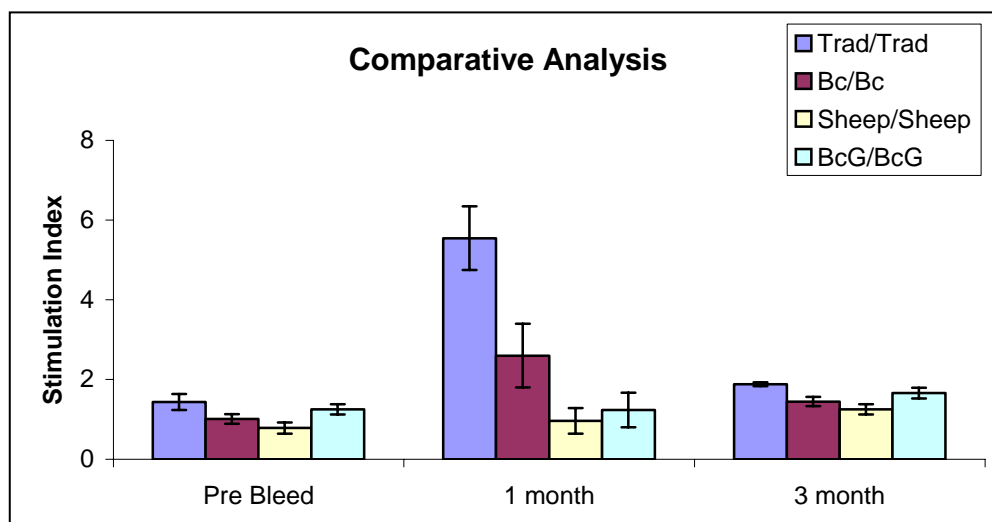


Figure 3: Memory response to the implantation of traditionally processed bovine bone (Trad), BioCleanse[®]-processed bovine bone (Bc), traditionally processed sheep bone (Sheep), and bovine bone processed by BioCleanse[®] followed by gamma irradiation (BcG).

Discussion

Results from this study clearly point to the presence of antigenic components in minimally processed (traditional) bone xenografts, refer to Figure 4. These components are present at levels that are capable of evoking a recall or memory response when peripheral blood mononuclear cells from the recipients were tested. The study also indicated that the BioCleanse[®] process was able to significantly reduce these



antigenic elements to levels incapable of eliciting a significant recall response. The recall responses measured in these studies appear to be of a transient nature as levels declined to pre-bleed or background levels by 3 months.

These results suggest that the antigenic elements associated with these grafts (as limited by this model) can influence the early rather than later phases of remodeling. This hypothesis is strongly supported by the quantitative histopathology report from the study. For example, evidence of hypercellularity associated with an inflammatory phenotype was significant at earlier time points (12 weeks) in the traditionally processed bovine implants, and subsided with time.

Conversely, bone in-growth into the implant was lowest in the traditionally processed bovine bone implants at 12 weeks, which increased to levels comparable to the other groups at 24 weeks. Taken together with the antigenicity data, it appears that residual donor material in the implants (expected in the traditionally processed bovine bone) evoked an inflammatory immune response, which adversely affected bone in-growth into the implants. As this response subsided with time, the remodeling efficiency in these implants was able to approach levels seen in controls (allografts). On the other hand, BioCleanse[®] processed bovine xenografts by virtue of depleted donor residues, was able to be remodeled at a pace comparable to allografts.

Conclusion

Results from this and the parent study demonstrate that residual donor material can influence bone graft remodeling in this aggressive xenograft (bovine bone implants in sheep) model. Treatment of bovine bone by the BioCleanse[®] Tissue Sterilization Process, with its known ability to remove residual endogenous material, rendered these xenografts to remodel as efficiently as an allograft.

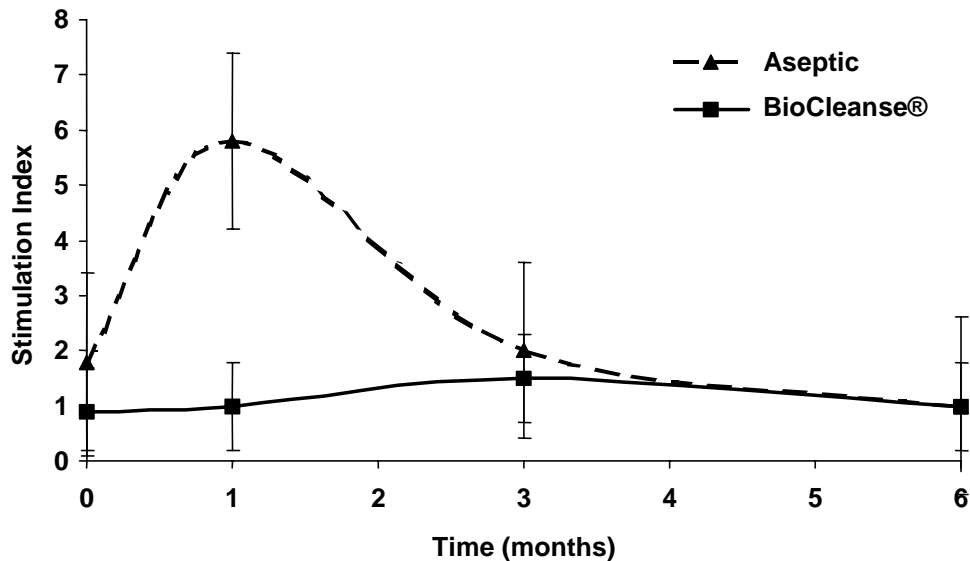


Figure 4: Comparison of the antigenic response elicited by the untreated vs. BioCleanse[®] treated tissue at 1, 3, and 6 months post-implantation.



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