Biomechanical Integrity of Human Allograft Bone After Sterilization

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Introduction:

Musculoskeletal allografts have been used extensively for a variety of orthopaedic procedures. Allograft does have the potential, however, to transmit a variety of pathogens. Two current sterilization methods widely used in the tissue banking industry are ethylene oxide (ETO) treatment and gamma irradiation. ETO has been shown to adversely affect the biologic incorporation of allograft tissue. Gamma irradiation has been shown to have an adverse effect on the mechanical properties of tissue at certain doses. A novel sterilization process for allografts, the BioCleanse® Tissue Sterilization Process, has been implemented that addresses the potential for disease transmission. Two studies, both using the BioCleanse process, were performed that examined the effects of various treatment regimes on the biomechanical properties of bone. The first study investigated effects of the BioCleanse process on the mechanical properties of bone. The second study evaluated bone through various processes, including the BioCleanse process combined with a terminal sterilization step in the final packaging.

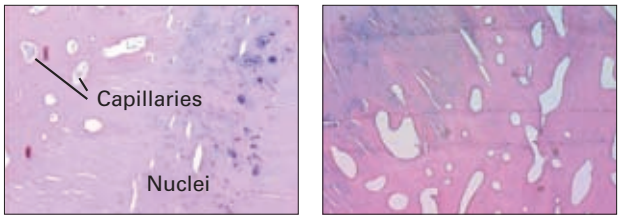

Figure 2. Histological analysis shows complete removal of cellular debris from bone. Capillaries and nuclei are present in aseptically treated bone. Bone treated through the BioCleanse® process shows no remnants of blood and cells.

Materials and Methods:

Source and Specimen Preparation

The first study used bone pins from seven male donors with an average age of 69 years (range 40 to 92 years). The second study had five male donors with an average age of 63 years (range 21 to 88 years) and one female donor 59 years of age.

Figure 1. Studies found that after 5 minutes contact time (normal cycle: 218 minutes), The BioCleanse® process completely perfused inner matrices of cortical and cancellous bone with sterilants.

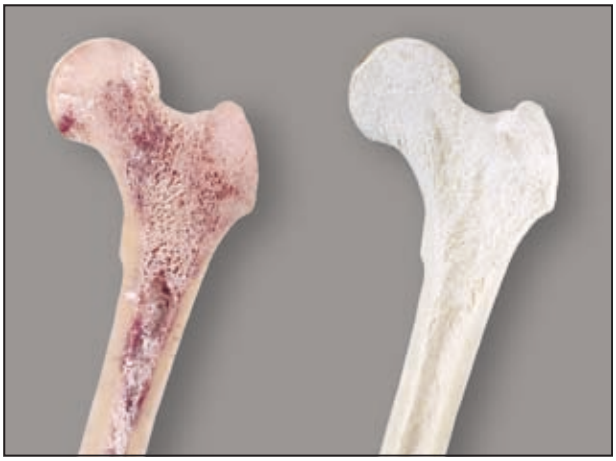

Figure 3. The BioCleanse® system sterilizes tissue using a complex, proprietary combination of mechanical and chemical processes, working in conjunction with each other. The mechanical component applies oscillating positive and negative pressure in the presence of the chemical agents (including detergents and sterilants), which gently perfuse the tissue. This combination removes blood and lipids, and inactivates or removes pathogenic microorganisms. Repeated rinses throughout the process remove debris, and final rinses remove residual chemicals, leaving the tissue biocompatible.

Test	Dimensions		Density (g/cm^3)[Y]	Failure Stress Equations[Y]
	Diameter (mm)	Length (mm)		
Axial Compression	3 - 6	5 - 7	$\frac{m}{\pi d^2 L/4(1000)}$	$\frac{FL}{\pi(d/2)^2}$
Diametral Compression	3 - 6	5 - 7	$\frac{m}{\pi d^2 L/4(1000)}$	$\frac{2 \times FL}{\pi dL}$
Shear	2.00 - 2.19	17.6 - 27.2	$\frac{m}{\pi d^2 L/4(1000)}$	$\frac{2 \times FL}{\pi d^2}$

[Y] The variables defined are mass (m), failure load (FL), diameter (d), and length (L) of the bone pins.

Table 1. Human cortical bone was machined into cylindrical pins for mechanical testing. The long axis of the pins was parallel to the long axis of the bone. Prior to mechanical testing, the mass, diameter and length were determined. The density was determined by assuming the volume of a perfect cylinder and the mass of the specimen.

Treatment Groups

In the first study, BioCleanse samples are compared to untreated samples. The second study has five treatment groups (Table 2). After machining, group 1 (control) was packaged and maintained frozen until testing. The remaining sets of specimens were treated through the BioCleanse process. Groups 2 and 3 were packaged and run through Sterrad® 200; Sterrad is a sterilization process that employs low temperature, plasma-potentiated vapor phase hydrogen peroxide. Group 4 was packaged and then irradiated (2.5-3.2 Mrad) while maintained on dry ice. All treatment groups were rehydrated except group 2.

Treatment Group	BioCleanse™ Phase I	Machining	BioCleanse™ Phase II	Lyophilization	Sterrad® 200 (2x)	Irradiation	Rehydration
1				X			X
2	X	X	X	X	X		
3	X	X	X	X	X		X
4	X	X	X	X		X	X
5	X	X	X	X			X

Table 2. The production process for the mechanical test specimens. The treated group in each study was compared to their respective control group.

Statistical Analysis

In the first study, ANOVA analysis was used to determine if the BioCleanse process had a significant ($p<0.05$) effect on the failure stress of bone. A two-sample t-test was used to examine the effects of the treatment groups in the second study. A significant difference in strength was considered to be greater than 15% reduction in load bearing capacity of the material when compared to controls.

Tests	FIRST STUDY (ANOVA)			
	Untreated		BioCleanse™	
	Samples	Failure Load (MPa)	Samples	Failure Load (MPa)
Axial	66	169 ± 21	60	167 ± 28
Diametral	94	25 ± 4	60	24 ± 6
Shear	66	62 ± 9	64	61 ± 9

Tests	SECOND STUDY (2-SAMPLE T-TEST)										Literature
	Untreated (1)		Sterrad® 2x (2)		Sterrad® 2x rehydrated (3)		Irradiated (4)		BioCleanse™ (5)		
	Samples	Failure Load (MPa)	Samples	Failure Load (MPa)	Samples	Failure Load (MPa)	Samples	Failure Load (MPa)	Samples	Failure Load (MPa)	
Axial	14	193 ± 19	14	278 ± 84	14	201 ± 38	14	194 ± 30	14	183 ± 18	131 - 224
Diametral	49	29 ± 5	49	37 ± 9	49	27 ± 7	49	26 ± 7	49	27 ± 6	9.9 - 56
Shear	30	63 ± 9	30	64 ± 10	30	61 ± 8	30	63 ± 10	30	56 ± 9	53 - 70

Table 3. Comparison of failure stresses of the two studies and values found in literature.

Results:

The BioCleanse process in the first study did not indicate a significant ($p>0.05$) effect of treatment on the failure stress of bone for all three modes of loading. The second study shows no significant difference in failure stress for all groups except the non-rehydrated Sterrad® 200 group. See Table 3.

Conclusions:

The risks to recipients from bacterial and viral contamination of aseptically processed tissue represent a rare but important public health problem. This problem can be addressed through application of BioCleanse processing to sterilize tissue without compromising biomechanical properties.

Physical and Mechanical Testing

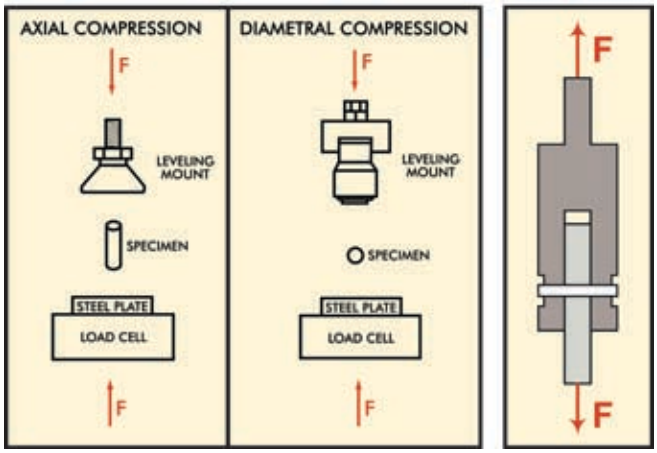

Figure 4. Testing was performed according to the proposed ASTM standard "Mechanical Testing of Bone or Bone Functional Replacements." The tests were performed on the MTS 850 Bionix. The failure load was recorded and used to calculate the failure stress (Table 1).