A NEW PROCESS FOR VIRAL CLEARANCE OF CORTICAL BONE ALLOGRAFTS AND THE EFFECTS ON THE MECHANICAL PROPERTIES AND OSTEOINDUCTIVITY

+DePaula, C A; +Mahony, D J; +Sunwoo, M H, +Gertzman, A A, *Dunn M G
+Musculoskeletal Transplant Foundation, Edison, NJ; *UMDNJ-RWJMS, New Brunswick, NJ

INTRODUCTION: Allografts are vital for bone stock deficiencies that occur during orthopaedic trauma, joint reconstruction, or other reconstructive procedures. The main issues for an orthopaedic allograft are the retention of strength, the retention of biologic factors, and the reduction of risk of disease transmission. The first two should not be affected by processing, while processing should eliminate the risk for disease transmission. Hydrogen peroxide (H_2O_2) is an oxidizing chemical used to process bone allografts with the potential to eradicate microorganisms and viruses that could potentially compromise osteoinductivity and bone structural proteins. This research tests the effects of tissue processing on the mechanical properties, osteoinductivity, and viral clearance of cortical bone allografts, under worst-case processing conditions.

METHODS: Processing consists of a nonionic detergent soak, a H_2O_2 soak, and a 70% alcohol (ethanol + isopropanol) soak, all under temperature controlled sonication (40kHz). **Mechanical:** Samples were processed as control (0-hour H_2O_2 treatment, with no sonication) or treatment (5-hour H_2O_2 treatment). Compression cylinders (5.3-mm * 5.3-mm) were fabricated from human femurs (age 39M & 61F) oriented longitudinally and transversely, and were preserved both frozen (-70C) and freeze-dried (N=8 for all groups). Freeze dried samples were rehydrated for at least 1-hour prior to testing and frozen samples were soaked for at least 15-minutes prior to testing, both in normal saline. Samples were loaded to failure in uniaxial compression at a strain rate of 0.01 sec^{-1}[1], maximum and yield stress were calculated. Impact specimens were fabricated into anterior cervical fusion (ACF) allografts from fibulas (age 46M, 21M, 60M, & 62M), and were preserved both frozen and freeze-dried (N=5 for all groups). Samples were secured into a custom fixture using 3 N-m of torque and impacted starting at 5-cm with 1-cm increments, using an ACF impact tool, and an 841-g carriage, until failure. Total kinetic energy at failure was calculated for each ACF. **Osteoinductivity:** Cortical bone from three donors (37M, 49M, 58F) was processed using a control process, 0 h H_2O_2 with no sonication, a treatment process with 1 h, 3 h, and 5 h of H_2O_2 treatment, and a negative control. After processing, the bone was ground, demineralized, and prepared into 32% DBM in a hyaluronan carrier. Samples (15 mg) were implanted bilaterally into the hamstring muscle in an athymic mouse model [2], approved by the UMDNJ animal care and use committee. Implants were evaluated histologically after 28 days [3]. **Viral Clearance:** Cortical bone samples were processed with a 1 h H_2O_2 step. Samples were subjected to the previous steps of the process. For each of six representative viruses, for each step of the process, samples were spiked with a virus suspension, and subjected to the given treatment step, while a control was subjected to an inert, zero-time, but equal-volume version of the same step. Supernatant was recovered from these samples, neutralized (where appropriate), and assayed for viral activity, using plaque and similar assays. Viral reduction for each virus for each step was calculated as the difference between the viral titer of the control, and the viral titer of the test sample at the full cycle time for the given treatment step. Results are the sum of the log reductions for all treatment steps for each virus.

RESULTS: Mechanical: The results of the compression testing show no significant differences between the control and treatment group maximum stress data (Table 1). Results of the impact testing revealed no significant differences between the control and treatment groups (Table not shown; the means (standard deviation) are: control = 49.8 (45.7); treatment = 35.2 (22.6)). **Osteoinductivity:** Hydrogen peroxide cleaning had a statistically significant effect on osteoinductivity, giving a linear decrease with increasing peroxide time (Figure 1). The mean (SD) osteoinductivity scores were 3.65 (0.49), 3.04 (0.97), 2.57 (1.36), 1.47 (1.10) for 0 h, 1 h, 3 h, and 5 h H_2O_2 treatment times, respectively. The negative control score was zero. Compared to the control (0 h), the 1 h score was not significantly different (p=0.113), and the 3 h and the 5 h scores were significantly different (p=0.045 & p=0.0001, respectively). **Viral Clearance:** The results of the viral clearance study demonstrate that processing the cortical bone allografts in the nonionic detergent, H_2O_2, and alcohol gives viral reductions greater than 6 logs in all cases except the PPV virus (Table 2).

DISCUSSION: Processing with a 5 h H_2O_2 soak does not affect the compression strength of cortical bone allografts. Likewise, the impact data did not show any statistical differences, however the standard deviations for this data are high. The osteoinductivity score for the 1 h H_2O_2 treatment time is favorable, because no statistical decrease was seen. The 3 h and 5 h treatment times were undesirable, as they caused statistically significant decreases in osteoinductivity. The viral clearance result verifies that the risk for disease transmission can be greatly reduced or eliminated by processing, beyond standard donor testing and screening procedures. Overall, these results demonstrate that it is possible to clean cortical bone allografts without causing a reduction in mechanical strength or a significant loss in osteoinductivity, while at the same time diminishing the risks of disease transmission.

Table 1: Compression maximum stress data (MPa) comparing the control groups to the 5-hour H_2O_2 treatment test groups

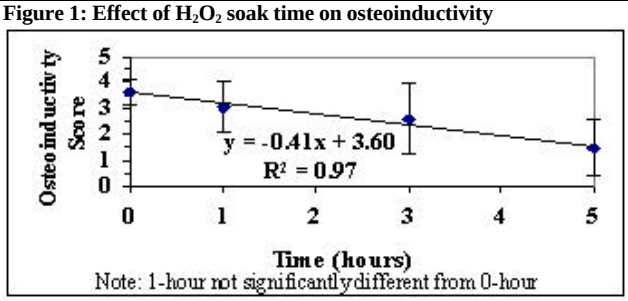
Storage	Donor Info	Tissue Orientation					
		Longitudinal			Transverse		
		Control	Test	Pr(F)	Control	Test	Pr(F)
Frozen	39m	164 (7)	159 (9)	0.25	128 (9)	119 (10)	0.07
	61f	156 (5)	159 (8)	0.42	124 (11)	121 (9)	0.52
Freeze-Dried	39m	219 (27)	222 (27)	0.82	153 (20)	167 (25)	0.24
	61f	206 (27)	202 (38)	0.82	127 (15)	117 (15)	0.21

Means are presented with their corresponding standard deviations in parentheses. For all groups, N = 8. The probability that the means are equal is given as Pr(F). Values < 0.05 are considered statistically different.

Table 2: Total viral clearance in cortical bone allograft due to processing in detergent, 1-hour hydrogen peroxide, and alcohol

Virus	RNA or DNA	Model for	Total Log Reduction of Virus
Enveloped Viruses			
BVDV (bovine viral diarrhea)	RNA	Hepatitis C (HCV)	>10.62
HIV (human immunodeficiency)	RNA	HIV	>15.22
PrV (Pseudorabies)	DNA	CMV/Herpes	>12.23
Non-Enveloped Viruses			
HAV (Hepatitis A)	RNA	HAV	>6.46
Polio	RNA	Polio/pico-rnaviridae	>10.96
PPV (porcine parvovirus)	DNA	Human parvovirus B19	2.57

Figure 1: Effect of H_2O_2 soak time on osteoinductivity



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