PREPARATION AND REHYDRATION EFFECTS ON COMPRESSIVE PROPERTIES OF CORNERSTONE ASR CERVICAL SPINE ALLOGRAFTS

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INTRODUCTION

The overall goal of this project was to determine the compressive properties of an anterior cervical interbody allograft subject to two preparation processes: (1) freeze dried and (2) frozen, and three rehydration processes: (1) no rehydration, (2) 30 s rehydration, and (3) 24 hour rehydration.

MATERIALS AND METHODS

Graft Description and Preparation. Cornerstone[1] ASR (Assembled Special Reserve) cervical spine allografts were supplied by RTI. Briefly, each ASR is comprised of a trabecular bone center assembled between lateral cortical bone plates via two cortical bone transverse pins (**Figure 1**). Machined ridges augment the cranial and caudal faces, and the anterior face is machined into a portion of a cylindrical surface Each specimen was randomly assigned to either the no rehydration group, the 30 s rehydration group, or the 24 hour rehydration group. Rehydration was accomplished by placing each specimen in phosphate buffered saline for the prescribed duration. Due to a power outage, the frozen-24 hour rehydration specimens for which test data were available were rehydrated for 26.5 hours.

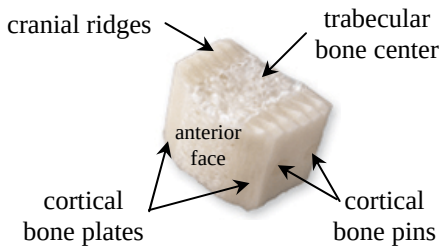

Figure 1. Cornerstone ASR cervical spine interbody allograft.

Specimen Dimensions. The dimensions of each specimen were measured prior to rehydration and/or mechanical testing with digital calipers. The dimensions measured (**Figure 2**) were the height h, from the cranial peaks to the caudal peaks; the width w, from the left lateral wall to right lateral wall; the depth d, from the most anterior margin to the posterior wall; the chord depth c, from the most anterior margin to the intersection of the anterior radius with the lateral walls; the cortical plate thickness t, as the average of the left plate thickness t_l and the right plate thickness t_r. The cortical bone area A_c and trabecular bone area A_t in the transverse plane of each specimen were computed from the measured dimensions, geometric formulas, and an assumed anterior radius of 11 mm (as supplied by RTI). The measured dimensions and computed areas are supplied in the **APPENDIX** for the freeze dried (**Table A1**) and frozen (**Table A3**) specimens.

Compression Testing. Compression testing was performed in a displacement controlled materials testing machine (InstruMet). Each specimen was placed between a fixed caudal platen and a self-leveling cranial platen within a "guided" compression fixture (**Figure 3**). The base of

[1] Registered trademark of Regeneration Technologies (RTI).

a linear extensometer was magnetically mounted to the crosshead, and the extensometer tip was placed in contact with the fixture near the caudal platen to eliminate fixture and test machine compliance. Each specimen was centered on the platen and then pre-loaded to 50 N prior to the application of a quasi-static crosshead displacement rate of 0.01 mm/s to failure. Load, extensometer displacement, and crosshead displacement was acquired at 1 Hz throughout each test.

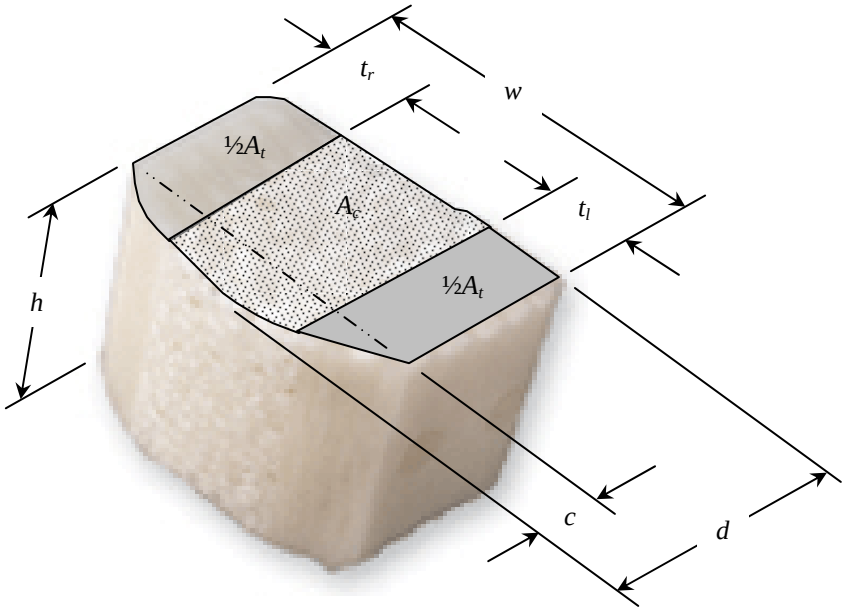

Figure 2. Dimensions measured on each specimen.

Mechanical Properties. The extensometer stiffness k_e [N/mm] for each specimen was determined from the applied compressive load as measured by the load cell and the graft compression as measured by the extensometer. The crosshead stiffness k_c [N/mm] for each specimen was determined from the graft compression as measured by the internal displacement transducer of the testing machine. Each stiffness of each specimen was determined from the linear portion of the applied compressive load-graft compression data, which was generally between 0.4 mm and 0.6 mm of graft compression as measured by the extensometer.

The extensometer modulus E_e [MPa = N/mm^2] for each specimen was determined from the linear portion of the load-compression data using the linear spring law

$$F = k_e \Delta h$$

where F is the applied compressive load and Δh is the graft compression. Dividing both sides of this expression by the original total graft area and multiplying the right hand side by unity in the form of the ratio of the original graft height to itself yields Hooke's Law in the form

$$\sigma = \frac{F}{A_c + A_t} = \frac{k_e h}{A_c + A_t}\frac{\Delta h}{h} = E_e \varepsilon$$

where σ and ε are graft stress and strain measures, respectively. The extensometer modulus can then be identified as

$$E_e = \frac{k_e h}{A_c + A_t}$$

The crosshead modulus E_c [MPa] can be similarly determined from the crosshead stiffness k_c. The ultimate load F_U [N] for each specimen was determined from the maximum load achieved during the test. An ultimate strength σ_U [MPa] for each specimen was determined by dividing the ultimate load by the original total graft area in the transverse plane

$$\sigma_U = \frac{F_U}{A_c + A_t}$$

The ultimate load, extensometer and crosshead stiffnesses, ultimate strength, and extensometer and crosshead moduli are supplied in the **APPENDIX** for the freeze dried (**Table A2**) and frozen (**Table A4**) specimens.

Statistical Analysis. Full interaction analyses of variance (ANOVAs) were performed to test the hypothesis that the mean cortical or trabecular bone areas and the mean ultimate strength or extensometer modulus of the freeze dried and frozen specimens were equal depending on the rehydration duration. Linear regression analyses were performed to determine if a correlation existed between cortical and trabecular areas or ultimate strengths and extensometer moduli. All statistical analyses were performed with commercially available software (StatView; SAS Institute; Cary, NC).

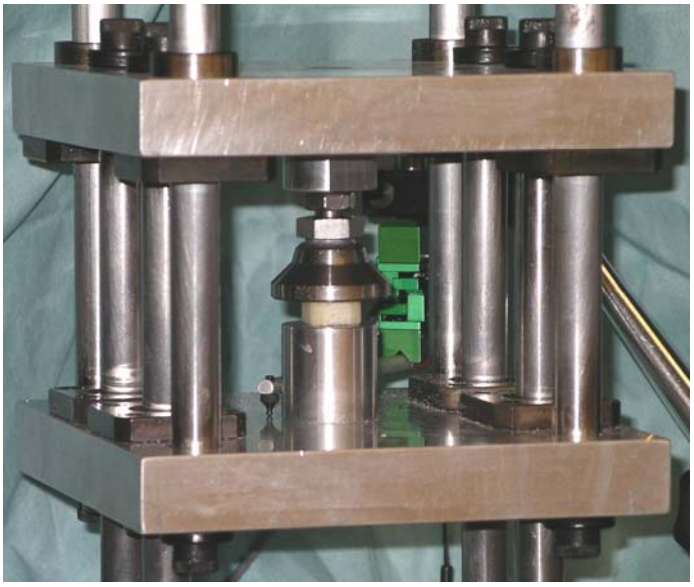

Figure 3. ASR allograft within guided compression fixture during testing.

RESULTS

The cortical or trabecular bone mean areas were not significantly different with regard to preparation and rehydration duration. The cortical bone mean (± standard deviation) areas were 72 mm^2 ± 5.2 mm^2 for the freeze dried and 73 mm^2 ± 5.2 mm^2 for the frozen specimens. The trabecular bone mean areas were 70 mm^2 ± 4.4 mm^2 for the freeze dried and 72 mm^2 ± 5.1

mm^2 for the frozen specimens. Cortical and trabecular bone areas were significantly and negatively correlated ($R^2 = 0.713$).

The extensometer moduli (**Table 1**) were significantly different ($P = 0.0040$) depending on preparation and rehydration duration. The mean extensometer moduli were greater for the freeze dried specimens compared to the frozen specimens for no rehydration and for a rehydration duration of 30 s. The mean extensometer modulus was greater for the frozen specimens compared to the freeze dried specimens for a rehydration duration of 24 hours.

The ultimate strengths (**Table 1**) were significantly different ($P = 0.0044$) depending on preparation and rehydration duration. The mean ultimate strengths were greater for the freeze dried specimens compared to the frozen specimens for no rehydration and for a rehydration duration of 30 s. The mean ultimate strength was greater for the frozen specimens compared to the freeze dried specimens for a rehydration duration of 24 hours. Extensometer moduli and ultimate strengths were significantly and positively correlated ($R^2 = 0.718$)

Preparation	Rehydration Duration	Extensometer Modulus (MPa)	Ultimate Strength (MPa)
Freeze Dried	0 s	434 ± 121	46 ± 7.8
	30 s	416 ± 103	45 ± 11
	24 hours	288 ± 72	34 ± 7.9
Frozen	0 s	328 ± 49	36 ± 4.5
	30 s	324 ± 62	34 ± 5.2
	24 hours	355 ± 63	37 ± 5.7

Table 1. Mean ± standard deviation values of extensometer moduli and ultimate strengths for the freeze dried and frozen specimens split by rehydration duration.

CONCLUSIONS

Long term rehydration, as would occur *in vivo*, of freeze dried Cornerstone ASR allografts caused a 32% reduction in stiffness (extensometer modulus) and a 26% reduction in (ultimate) strength compared to no or short term rehydration. Long term rehydration of frozen Cornerstone ASR allografts caused a 9% increase in stiffness (extensometer modulus) and a 7% increase in (ultimate) strength compared to no or short term rehydration. For long term rehydration, freeze dried Cornerstone ASR allografts were 19% less stiff (extensometer modulus) and 9% weaker (ultimate strength) compared to frozen Cornerstone ASR allografts.

ACKNOWLEDGEMENTS

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APPENDIX

Specimen	Rehydration Duration	Height, h (mm)	Depth, d (mm)	Width, w (mm)	Chord Depth, c (mm)	Lateral Plate Width, t (mm)	Cortical Bone Area, A_c (mm^2)	Trabecular Bone Area, A_t (mm^2)
DN7-1	0	6.7	10.7	14.0	2.9	3.6	72.5	72.8
DN7-2	0	6.8	10.7	13.7	2.7	3.7	75.7	67.4
DN7-3	0	7.0	10.7	13.9	2.9	3.4	67.7	76.0
DN7-4	0	7.0	10.5	13.8	2.7	3.3	65.2	75.6
DN7-5	0	6.9	10.7	13.8	3.3	3.6	69.2	70.6
DN7-6	0	6.7	10.6	13.7	2.5	3.5	71.7	71.0
DN8-1	0	7.8	10.7	13.8	3.0	4.0	80.7	62.1
DN8-2	0	8.1	10.9	13.9	2.8	3.9	81.7	66.5
DN8-3	0	8.0	10.8	13.5	2.8	3.5	70.6	70.2
DN8-4	0	7.9	10.6	13.7	2.9	3.7	73.5	66.8
DS7-1	30 s	6.9	10.5	13.7	2.9	3.7	72.8	66.2
DS7-2	30 s	7.0	10.5	13.9	2.5	3.2	64.3	78.8
DS7-3	30 s	7.2	9.9	14.0	1.7	3.1	63.4	77.2
DS7-4	30 s	6.9	10.6	13.6	2.7	3.6	72.5	67.8
DS7-5	30 s	6.9	10.6	13.7	3.0	3.4	65.9	73.1
DS7-6	30 s	7.3	10.7	13.6	2.8	3.5	70.1	70.6
DS8-1	30 s	8.1	10.7	13.8	2.5	3.4	70.2	74.9
DS8-2	30 s	7.9	10.7	13.8	3.0	3.3	64.5	77.0
DS8-3	30 s	7.8	10.5	13.8	2.5	3.7	75.9	67.2
DS8-4	30 s	8.0	10.8	13.4	2.9	3.6	72.1	67.0
DL7-1	24 hours	6.9	10.7	13.7	2.9	3.8	76.6	65.3
DL7-2	24 hours	7.5	10.8	13.8	2.8	3.9	80.7	64.8
DL7-3	24 hours	6.9	10.8	13.8	3.0	3.9	79.2	64.8
DL7-4	24 hours	6.7	10.5	13.5	2.3	3.7	76.8	64.1
DL7-5	24 hours	6.8	10.7	13.8	2.7	3.8	78.3	66.3
DL7-6	24 hours	7.1	10.6	13.7	2.5	3.5	71.7	71.0
DL8-1	24 hours	8.0	10.7	13.7	2.7	3.5	71.0	71.7
DL8-2	24 hours	7.9	10.6	13.8	3.1	3.5	67.7	72.1
DL8-3	24 hours	7.8	10.5	13.8	3.0	3.4	65.4	73.5
DL8-4	24 hours	7.9	10.4	13.9	3.1	3.6	68.7	69.7

Table A1. Freeze dried specimen measured dimensions and computed areas.

Specimen	Rehydration Duration	Ultimate Load, F_U (N)	Extensometer Stiffness, k_e (N/mm)	Crosshead Stiffness, k_c (N/mm)	Ultimate Strength, σ_U (MPa)	Extensometer Modulus, E_e (MPa)	Crosshead Modulus, E_c (MPa)
DN7-1	0	6,316	10,452	5,824	43.5	482	269
DN7-2	0	7,106	7,913	6,007	49.6	376	285
DN7-3	0	4,252	5,546	3,752	29.6	270	183
DN7-4	0	5,761	6,127	4,335	40.9	305	216
DN7-5	0	8,055	12,999	7,421	57.6	641	366
DN7-6	0	6,144	7,940	5,756	43.0	373	270
DN8-1	0	7,218	11,080	7,354	50.5	605	402
DN8-2	0	6,289	6,657	3,921	42.4	364	214
DN8-3	0	7,012	8,476	5,124	49.8	481	291
DN8-4	0	7,385	7,774	5,064	52.6	438	285
DS7-1	30 s	6,276	10,111	6,384	45.2	502	317
DS7-2	30 s	8,020	7,871	5,148	56.1	385	252
DS7-3	30 s	3,877	4,593	3,563	27.6	235	182
DS7-4	30 s	6,430	6,837	4,697	45.8	336	231
DS7-5	30 s	4,078	5,780	4,219	29.3	287	209
DS7-6	30 s	6,083	8,014	5,763	43.2	416	299
DS8-1	30 s	6,142	8,396	5,986	42.3	469	334
DS8-2	30 s	7,972	8,833	5,789	56.3	493	323
DS8-3	30 s	6,494	9,200	5,958	45.4	501	325
DS8-4	30 s	8,217	9,369	6,174	59.1	539	355
DL7-1	24 hours	5,619	6,455	4,479	39.6	314	218
DL7-2	24 hours	2,695	2,987	2,527	18.5	154	130
DL7-3	24 hours	5,548	6,719	4,713	38.5	322	226
DL7-4	24 hours	4,635	5,829	4,237	32.9	277	202
DL7-5	24 hours	5,560	6,911	4,855	38.4	325	228
DL7-6	24 hours	3,300	3,836	3,046	23.1	191	152
DL8-1	24 hours	6,042	6,480	4,625	42.3	363	259
DL8-2	24 hours	5,746	6,825	4,862	41.1	386	275
DL8-3	24 hours	4,148	5,026	3,858	29.9	282	217
DL8-4	24 hours	4,698	4,650	3,634	33.9	265	207

Table A2. Freeze dried specimen mechanical properties.

Specimen	Rehydration Duration	Height, h (mm)	Depth, d (mm)	Width, w (mm)	Chord Depth, c (mm)	Lateral Plate Width, t (mm)	Cortical Bone Area, A_c (mm^2)	Trabecular Bone Area, A_t (mm^2)
FN7-1	0	6.8	10.6	13.9	3.1	3.8	74.7	66.8
FN7-2	0	7.1	10.7	14.0	3.3	3.6	69.6	72.8
FN7-3	0	7.1	10.8	14.1	2.8	3.7	76.6	72.4
FN7-4	0	7.0	10.8	14.0	3.1	3.8	76.5	69.1
FN7-5	0	7.2	10.7	13.7	2.9	3.9	78.9	63.1
FN7-6	0	7.0	10.9	14.1	3.0	3.6	73.4	75.2
FN8-1	0	8.2	11.1	13.5	3.1	3.9	80.1	63.3
FN8-2	0	8.1	10.8	13.9	3.1	3.8	76.3	68.0
FN8-3	0	8.2	10.7	14.0	3.2	3.5	68.1	74.9
FN8-4	0	7.8	10.7	13.9	3.3	3.5	67.2	73.8
FS7-1	30 s	7.1	10.9	13.9	3.1	3.7	74.7	70.9
FS7-2	30 s	7.0	10.7	13.9	3.0	3.8	76.3	67.4
FS7-3	30 s	7.0	10.9	14.0	2.8	3.4	69.9	78.5
FS7-4	30 s	7.0	10.7	14.0	2.7	3.6	73.9	72.8
FS7-5	30 s	7.1	10.7	13.8	2.1	3.7	80.4	68.5
FS7-6	30 s	7.2	10.6	14.0	2.4	3.4	70.6	76.3
FS8-1	30 s	8.0	10.8	14.0	3.1	3.5	69.5	75.6
FS8-2	30 s	8.2	10.8	13.9	2.4	3.6	76.6	72.4
FS8-3	30 s	8.0	10.7	14.0	2.6	3.6	74.7	72.8
FS8-4	30 s	7.9	10.9	14.0	3.3	3.6	71.1	74.1
FL7-1	24 hours	7.0	10.8	14.0	3.1	3.7	74.1	71.3
FL7-2	26.5 hours	6.8	10.6	13.8	2.8	3.8	76.8	65.7
FL7-3	26.5 hours	6.9	10.6	13.8	2.8	3.9	79.2	63.6
FL7-4	26.5 hours	6.7	10.8	14.0	3.2	3.8	75.7	69.1
FL7-5	26.5 hours	7.1	10.8	13.9	3.1	3.7	73.9	70.2
FL7-6	26.5 hours	7.1	10.8	14.0	3.2	3.7	73.4	71.3
FL8-1	26.5 hours	7.9	10.8	14.0	3.0	3.5	70.2	75.6
FL8-2	26.5 hours	7.9	10.7	13.9	3.1	2.8	52.9	88.8
FL8-3	26.5 hours	8.2	11.0	13.8	3.1	3.7	75.2	70.4
FL8-4	26.5 hours	8.2	11.0	13.8	2.9	3.6	74.3	72.6

Table A3. Frozen specimen measured dimensions and computed areas.

Specimen	Rehydration Duration	Ultimate Load, F_U (N)	Extensometer Stiffness, k_e (N/mm)	Crosshead Stiffness, k_c (N/mm)	Ultimate Strength, σ_U (MPa)	Extensometer Modulus, E_e (MPa)	Crosshead Modulus, E_c (MPa)
FN7-1	0	4,463	5,753	4,446	31.5	276	214
FN7-2	0	4,952	5,321	3,934	34.8	265	196
FN7-3	0	6,319	7,130	5,073	42.4	340	242
FN7-4	0	5,433	7,961	5,358	37.3	383	258
FN7-5	0	5,389	6,589	4,816	37.9	334	244
FN7-6	0	5,053	6,121	4,507	34.0	288	212
FN8-1	0	5,702	6,928	4,904	39.8	396	281
FN8-2	0	3,967	5,186	3,962	27.5	291	222
FN8-3	0	4,514	5,501	4,341	31.6	316	249
FN8-4	0	5,435	7,042	5,096	38.5	390	282
FS7-1	30 s	5,172	5,914	4,362	35.5	289	213
FS7-2	30 s	5,008	7,214	5,210	34.9	351	254
FS7-3	30 s	5,203	7,181	5,305	35.1	339	250
FS7-4	30 s	5,442	6,961	5,028	37.1	332	240
FS7-5	30 s	5,090	6,784	4,941	34.2	324	236
FS7-6	30 s	3,126	3,764	3,014	21.3	185	148
FS8-1	30 s	4,565	5,044	4,012	31.5	278	221
FS8-2	30 s	4,922	6,726	4,939	33.0	370	272
FS8-3	30 s	5,563	7,466	5,282	37.7	405	287
FS8-4	30 s	5,885	6,807	5,142	40.5	370	280
FL7-1	24 hours	lost	lost	lost	lost	lost	lost
FL7-2	26.5 hours	6,061	2,899	4,928	42.5	lost	235
FL7-3	26.5 hours	4,027	6,182	4,600	28.2	299	222
FL7-4	26.5 hours	4,967	7,324	5,226	34.3	339	242
FL7-5	26.5 hours	5,060	5,643	4,161	35.1	278	205
FL7-6	26.5 hours	5,748	6,799	4,939	39.7	334	242
FL8-1	26.5 hours	5,383	7,024	5,041	36.9	381	273
FL8-2	26.5 hours	4,339	5,844	4,361	30.6	326	243
FL8-3	26.5 hours	6,389	7,313	5,161	43.9	412	291
FL8-4	26.5 hours	6,426	8,442	5,755	43.8	471	321

Note: Test data lost for specimen FL7-1 due to regional power outage; extensometer data lost for specimen FL7-2 due to possible contact with loading platen during test.

Table A4. Frozen specimen mechanical properties.