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## Rehydration improves the ductility of dry bone allografts

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1 Rehydration improves the ductility of dry bone allografts

2 **Short title: Mechanical properties of bone allografts**

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23 ethics statement: "N/A"

24

## 25 **Abstract:**

26 Processing of bone allografts improves infectious safety and allows storing bone substitutes at room  
27 temperature. The aim of this study was to compare mechanical properties of the processed  
28 Osteopure™ bone with fresh frozen bone. All the samples were pieces from femoral heads retrieved  
29 during hip arthroplasty operations. The processing includes chemical decellularization, drying and  
30 irradiation with 25 kGy.

31 Three types of samples were tested:

- 32 1. fresh frozen thawed wet,
- 33 2. dry non-rehydrated graft
- 34 3. dry rehydrated graft

35 In the 3-point bending test Young's modulus and stress at break yielded no significant difference  
36 among the 3 different sample groups. Rehydrating of the dry graft showed increased ductility in  
37 strain at break test compared with the other 2 groups ( $p=0.003$ ). In compression tests dry grafts had  
38 significantly higher maximum effective stress and apparent maximum deformation compared with  
39 the grafts of other groups ( $p<0.05$ ).

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41 Processed bone has almost similar mechanical properties compared with fresh frozen bone.  
42 However, rehydration of processed dry graft increases its ductility. These grafts may tolerate bending  
43 forces better before breakage.

44

## 45 **Introduction**

46 Clinical bone loss may require autograft which provides a scaffold and some viable  
47 cells. Autograft is considered a comparable gold standard in filling voids. There is  
48 morbidity in recovering autograft and there is a limit to the volume obtained (1–6).  
49 The allograft can be done without volume limitation. There is more experience with  
50 deeply frozen bone allografts. Formal processing of the allograft decreases the risk of  
51 transfer of infectious disease and allows the allograft to be stored at ambient  
52 temperature. Ideally processing should not change the mechanical properties  
53 compared to fresh bone (7,8).

54 The aim of this study was to compare the mechanical properties of fresh human bone  
55 with bone treated by a process like Osteopure, dry and rehydrated state as used in  
56 daily practice and define if there are differences in biomechanical properties.

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## 59 **Method**

60 The trabecular bone samples were recovered during a total hip arthroplasty for  
61 osteoarthritis as part of routine surgery. The tissues were obtained from the tissue  
62 bank "ostéobanque d'auvergne" (France) with consent of patients for a medical and  
63 research use of their tissue. No ethic committee was sought. Different types of  
64 samples were tested: a first group of anatomical specimens from "fresh" wet frozen  
65 and thawed at ambient temperature for 12 hours before carrying out the tests (not  
66 irradiated), a second group of samples "dry" having been subjected to the  
67 Osteopure™ (decellularization technique using chemical products for bone cleaning  
68 and dehydration with ethanol) including sterilization at 25 kGy and a third group  
69 "dry rehydrated" allograft processed similar to the second group immersed in saline  
70 for a period of 10 minutes as usually practiced in the operating room prior to  
71 implantation. Each group of samples was subjected to a series of tests to determine  
72 their mechanical properties. The geometry of samples depended on the type of  
73 mechanical test. The procedure includes decortication, cutting and primary cleaning,  
74 then automated chemical processing with solvent, detergent and urea, drying with  
75 ethanol, packaging and finally sterilization with beta irradiation.

76 A rectangular shape was used for the bending test (60x10x10 mm<sup>3</sup>) and for  
77 hardness test (20x10x10 mm<sup>3</sup>), a cubic geometry (10 mm<sup>3</sup>) was selected for the  
78 compression test.

## 79 **Mechanical tests**

80 Bending and compression tests were carried out on a traction device (MTS 20M)  
81 equipped with a 3-point bending platform (a range of 40 mm was used) and  
82 compression plates. The unit is controlled by software managing during the entire  
83 procedure of tests and results processing. A 500 daN force sensor was available for  
84 these destructive testing. Measuring the deformation was followed from the  
85 registration of the crosshead (resolution 100 μm). The speed of movement of the  
86 crosshead was set during the two tests at 3 mm / min.

87  
88 The 3-point bending test, leading to rupture of the sample, resulting in a stress-strain  
89 curve (Figure 1) which allows the determination of Young's modulus from the slope  
90 foot curve and measuring the stress and strain associated with the breaking point.  
91 During this test, the bone material is placed on two fixed supports; a third support in a  
92 central position, placed perpendicular to the horizontal plane defined by the sample is  
93 directed downward and the filler material until it breaks. We tested an average of 10  
94 pieces for each group.

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98 Figure 1: stress-strain curve of a fresh bone subjected to a 3-point bending test

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102 The shape of the stress-strain curve of a trabecular bone sample under compression  
103 (Figure 2) is specific to the mechanical behavior of this type of scaffold. For these  
104 materials, the section and the characteristic length of the apparent geometry of the  
105 specimen differs from the section and length of the resistive material. The scaffold  
106 consists of a lattice of materials and voids, the actual section being only  
107 characterized by the material. We tested an average of 10 pieces for each group.

108 Its mechanical behavior can be distinguished into 3 phases:

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- A first phase of resilient loading characterized by a homogeneous deformation of the architecture coupled to the edges of the bending mechanisms (buckling). Young's modulus ( $E$ ) or apparent modulus ( $E_{app}$ ) of the scaffold is determined during the first compression phase. It corresponds to the slope of pseudoelastic loading samples in this area. Also determined at this stage the apparent maximum stress ( $\sigma_{max(app)}$ ) corresponding to the strain limit value between the first and second phase and its associated distortion, the apparent maximum deformation ( $\epsilon_{max(app)}$ ).
  - A second phase characterized by a long almost constant stress plateau, is the collapse of the voids, similar if a screw is placed in the graft.
  - A third phase characterized by an increase in stress describing the Young's modulus of the material. It corresponds to a complete compaction of the trabecular voids. This occurs more or less rapidly depending on the porosity of the material. The set of edges and walls are in contact; porosities are all destroyed; only the material is compressed. This can be characterized for the corresponding phase, the densification module ( $E_{dens}$ ) and the corresponding level of deformation ( $\epsilon_{dens}$ ) reached. The denser the material, the quicker the porosities are filled and the quicker the densification phase occurs.

127

128 Figure 2: stress-strain curve of trabecular bone subjected to compression test

129 Lack of homogeneity of deformation fields and the random distribution of voids within  
130 a sample hardly make identifiable the actual resistant material. If the samples tend to  
131 have a similar volume, the resistant material may therefore be different from one  
132 sample to another.

133 During the test, to facilitate the analysis of results and enable a comparison of  
134 mechanical responses among different specimens, the results have been recorded in  
135 conventional stresses and strains, calculated from the apparent size of the samples.  
136 An automated procedure is used to extract the following mechanical parameters: the  
137 apparent modulus  $E_{app}$ , maximum apparent stress  $\sigma_{max(app)}$  and maximum apparent  
138 deformation  $\epsilon_{max(app)}$ .

139 The densification module ( $E_{dens}$ ) was not calculated, the upper limit of the force  
140 sensor was often reached during the third stage of compression test. The tests were

141 halted voluntarily in this third phase, between 50 and 70% deformation, since this limit  
142 has no clinical consequence, it is not reached with use of allograft in vivo.

143

144 The third mechanical test, the Shore A hardness test was performed on a Shore  
145 durometer fitted with a specific head that measure (figure 3). This test measures the  
146 surface hardness. We tested an average of 60 pieces of fresh bone, and an average  
147 of 90 pieces of dry and dry rehydrated bone.

148 Figure 3 : Shore A hardness test

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## 150 **Statistical analysis**

151 An ANOVA (analysis of variance) was used to study the dependence of the  
152 mechanical properties for the type of sample conditioning ("fresh" "dry" and "Dry  
153 rehydrated"). Results were considered significantly different at  $p < 0.05$ .

154 Determining each mechanical characteristic of a group of samples is derived from a  
155 minimum of 8 tests in exactly the same testing conditions.

156 In order to characterize the existence of possible correlations (or other linear  
157 relationship) between the measured mechanical quantities, a nonparametric  
158 Spearman correlation test was conducted. The variables (mechanical quantities) are  
159 tested 2 by 2, the correlation coefficient  $\rho$  is calculated with the significance level (p-  
160 value) (at the risk level of 5%). The intensity of the dependence between two  
161 variables is directly related to the value  $\rho$ . The correlation is important for  $|\rho| > 0.8$ , it is  
162 more moderate for  $0.7 < |\rho| < 0.8$ , for low values of  $0.6 < |\rho| < 0.7$  and non-significant  
163 below 0.6.

## 164 **Results**

165 The results of bend tests, compression and hardness are summarized in Table 1.

166 Table 1. Results of mechanical tests on groups of samples tested

167

168 The results of bending tests indicate that the Young's modulus values were not  
169 significantly different for the three groups tested ( $p = 0.502$ ) and an average value of  
170  $218.3 \pm 44.7$  MPa may be used for all samples. It is the same for the breaking stress  
171 ( $p = 0.983$ ), which leads ultimately to a value of  $9.49 \pm 1.88$  Mpa with the average of  
172 the three sample groups.

173 Strain at break point yielded interesting result. The measured value was broadly  
174 similar for groups "Fresh" and "Dry" but increased remarkably in the group  
175 "Rehydrated". The difference was statistically significant ( $p=0.003$ ). Rehydrated grafts  
176 appeared to be more ductile.

177 In compression test the value of deformation max showed no difference between the  
178 groups. However, statistical significant difference was seen in apparent modulus  
179 ( $p=0.038$ ) and in constraint max test ( $p=0.047$ ) when compared dry grafts with the  
180 other grafts

181 The values measured for the deformation  $\epsilon_{max}$  (app) associated with the maximum  
182 stress showed no difference at the same time ( $p = 0.949$ ) for the 3 groups tested.

183

184 The values in Shore hardness test were almost identical between the groups showing  
185 no statistical difference.

186 Tables 2 and 3 show the matrices of Spearman correlation coefficients between the  
187 various mechanical parameters measured during the bending and compression tests

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189 Table 2: matrices Spearman correlation coefficients between mechanical parameters  
190 measured during the bending test

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193 Table 3: matrices Spearman correlation coefficients between mechanical parameters  
194 measured during the compression test

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196 We note a clear correlation between variables "Young's modulus" and "tensile  
197 strength" of the bending test ( $\rho$  evolving between 0.738 and 0.917) and a perfectly  
198 similar dependence ( $\rho$  ranging from 0.714 - 0.895) between the parameters  
199 "apparent modulus" and "maximum effective stress" of the compression test.

200 Correlations tests result in a significant linear relationship between the Young's  
201 modulus and the stress at break in flexure on the one hand and the apparent  
202 modulus and maximum effective stress evaluated by compression test on the other  
203 hand.

## 204 Discussion



205 Our results are similar to those reported by J. and Halgrin. al.(9), in the case of  
206 compression tests conducted on trabecular bone samples with or without lipid and  
207 marrow removal. The authors demonstrate a significant decrease in the apparent  
208 modulus and apparent maximum stress for samples where lipid and marrow was  
209 absent. The fluid is pressurized in the center of the specimen, which has the effect of  
210 increasing the transverse load applied to the trabecular structure and damages faster  
211 all trabeculae, resulting in a collapse peak (peak before the second phase of the  
212 deformation) and a lowered strain plateau. It is also demonstrated by Ochoa with  
213 rigidity varying more than 30% following the removal of fluids.

214 Matrices Spearman correlation coefficients between the various mechanical  
215 parameters measured showed the correlation between modulus of elasticity and the  
216 tensile strength in bending and between the apparent modulus and the stress  
217 apparent maximum compression test; these correlations allows one to establish a  
218 linear relationship between the quantities concerned.

219 Ochoa et Poumara(10,11) have reported similar correlations between apparent  
220 modulus and maximum apparent stress in compression tests performed on  
221 trabecular bone specimens subjected to a different processing (cleaning and  
222 sterilization). Their works show that the relationship between these two factors  
223 characterizing the mechanical behavior of the bone; and remains valid irrespective of  
224 processing of the bone. Our results are in agreement with this work; in the case of  
225 compression tests, our conclusion is substantially identical regardless of the  
226 processing applied to the sample.

227 Other bone processes techniques also found similar results to ours with no  
228 difference between the frozen and processed bones (12) but bone mechanical  
229 properties can be affected by ionizing radiation (13). It was not found in our study, but  
230 we didn't compare treated bone not irradiated versus irradiated. We studied  
231 cancellous bone and not cortical, this can distort results.

232 The bending test seems to lead to different conclusions. As shown by the regression  
233 lines, the behavior of groups of samples "Fresh" and "Dry" are relatively similar but  
234 certain parameters are different from the "Dry rehydrated" group. These results  
235 confirm those from ANOVA study which indicated a very significant difference in  
236 terms of elongation at break (greater ductility of the group "Dry rehydrated" revealed  
237 by the bending test) (14). This point has clinical consequences and leads us to  
238 rehydrate bone especially if the graft undergoes deformation during the time of early

239 implantation. The allograft would tolerate more deformation before breaking if well  
240 hydrated.

241 The dry allograft will rehydrate with body fluids after implantation. It will change some  
242 of the biomechanical properties like strain to failure and maximum stress. Therefore,  
243 rehydration before implantation could be recommended if the graft is used as  
244 impaction graft or fixed by screws. Moreover, our compression tests show that the dry  
245 bone has a maximum effective stress and an apparent maximum deformation  
246 significantly higher compared to fresh bone. These values again become similar after  
247 rehydrating of dry bones.

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## 249 **Conclusion**

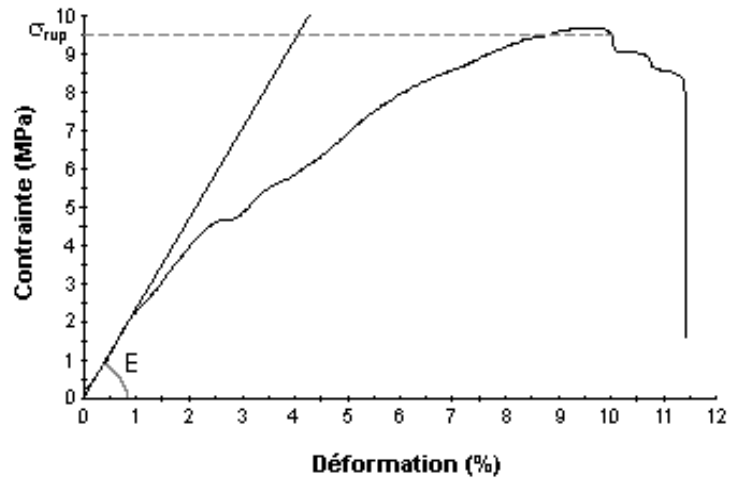
250 It appears that the processed bone by the Osteopure™ technique has mechanical  
251 properties substantially similar to fresh bone. Rehydrating samples does not modify  
252 the surface hardness but retrieves an apparent Young's modulus and a maximum  
253 stress apparently closer to that of fresh bones. Rehydration of the dry allografts as  
254 instructed by the processor improves ductility parameters (becomes greater) which  
255 means the bone can deform further before breaking.

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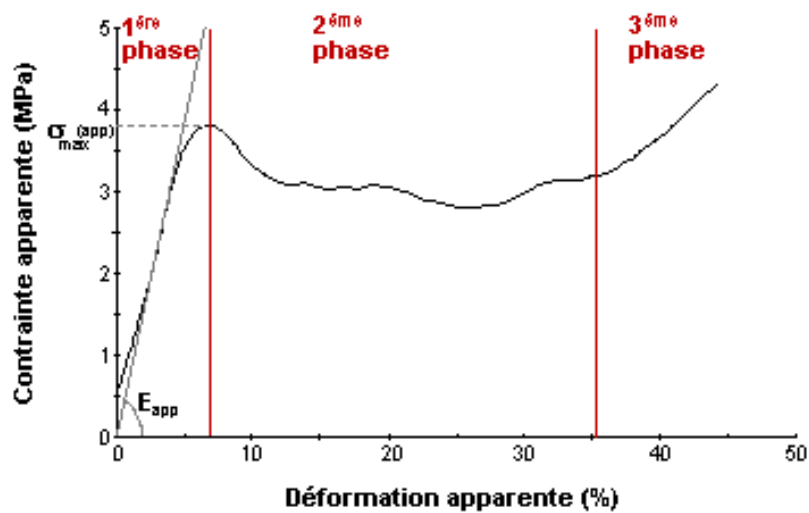
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Figure 1: stress-strain curve of a fresh bone subjected to a 3-point bending test



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Figure 2: stress-strain curve of trabecular bone subjected to compression test

Table 1. Results of mechanical tests on groups of samples tested

mechanical characterization	fresh bones	dry bones	Dry bones moistened	
<b>3-point bending</b>				
Young's modulus (MPa)	239.6 ± 78.2	233.2 ± 108.5	181.5 ± 80.4	p=0.502
Stress at break (MPa)	8.37 ± 3.30	8.89 ± 3.45	11.23 ± 4.12	p=0.983

Strain at break (%) 5.86± 1.46 5.34 ± 2.58 **10.82 ± 4.75** **p=0.003**

**Compression**

Apparent modulus (Mpa) 91.2 ± 15.7 **144.9 ± 37.8** 87.1 ± 41.5 **p=0.038**

Constraint max. (App) (Mpa) 4.51 ± 0.91 **7.31 ± 1.96** 4.30 ± 2.27 **p=0.047**

Max deformation. (App) (%) 8.39 ± 1.86 7.84 ± 3.61 8.20 ± 2.16 p=0.949

**Shore A hardness** 72.35± 2.04 73.85 ± 1.58 74.10 ± 2.36 p=0.522

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300 Table 2: matrices Spearman correlation coefficients between mechanical parameters measured  
301 during the bending test

302 (intensity of the correlation: red, high / blue, medium / white: not significant)

303

Os FRAIS				Os SEC				Os SEC réhumidifié			
	$\sigma_{break}(app)$ (MPa)	$\epsilon_{break}(app)$ (%)	E (MPa)		$\sigma_{break}(app)$ (MPa)	$\epsilon_{break}(app)$ (%)	E (MPa)		$\sigma_{break}(app)$ (MPa)	$\epsilon_{break}(app)$ (%)	E (MPa)
$\sigma_{break}(app)$ (MPa)	$\rho = 1$	$\rho = 0.539$ p-value: 0.054	$\rho = 0.855$ p-value<0.001	$\sigma_{break}(app)$ (MPa)	$\rho = 1$	$\rho = 0.071$ p-value: 0.433	$\rho = 0.738$ p-value: 0.018	$\sigma_{break}(app)$ (MPa)	$\rho = 1$	$\rho = -0.050$ p-value: 0.551	$\rho = 0.917$ p-value<0.001
$\epsilon_{break}(app)$ (%)		$\rho = 1$	$\rho = 0.588$ p-value: 0.037	$\epsilon_{break}(app)$ (%)		$\rho = 1$	$\rho = -0.595$ p-value: 0.940	$\epsilon_{break}(app)$ (%)		$\rho = 1$	$\rho = -0.417$ p-value: 0.868
E (MPa)			$\rho = 1$	E (MPa)			$\rho = 1$	E (MPa)			$\rho = 1$

304

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306 Table 3: matrices Spearman correlation coefficients between mechanical parameters measured  
307 during the compression test

308 (intensity of the correlation: red, high / blue, medium / white: not significant)

Os FRAIS				Os SEC				Os SEC réhumidifié			
	$\sigma_{max}(app)$ (MPa)	$\epsilon_{max}(app)$ (%)	$E_{app}$ (MPa)		$\sigma_{max}(app)$ (MPa)	$\epsilon_{max}(app)$ (%)	$E_{app}$ (MPa)		$\sigma_{max}(app)$ (MPa)	$\epsilon_{max}(app)$ (%)	$E_{app}$ (MPa)
$\sigma_{max}(app)$ (MPa)	$\rho = 1$	$\rho = 0.071$ p-value: 0.433	$\rho = 0.714$ p-value: 0.023	$\sigma_{max}(app)$ (MPa)	$\rho = 1$	$\rho = 0.583$ p-value: 0.05	$\rho = 0.733$ p-value: 0.012	$\sigma_{max}(app)$ (MPa)	$\rho = 1$	$\rho = 0.077$ p-value: 0.406	$\rho = 0.895$ p-value<0.001
$\epsilon_{max}(app)$ (%)		$\rho = 1$	$\rho = -0.024$ p-value: 0.522	$\epsilon_{max}(app)$ (%)		$\rho = 1$	$\rho = 0.217$ p-value: 0.288	$\epsilon_{max}(app)$ (%)		$\rho = 1$	$\rho = -0.245$ p-value: 0.778
$E_{app}$ (MPa)			$\rho = 1$	$E_{app}$ (MPa)			$\rho = 1$	$E_{app}$ (MPa)			$\rho = 1$

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310