Rehydration improves the ductility of dry bone allografts

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Short title: Mechanical properties of bone allografts

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Abstract:

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

Three types of samples were tested:

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

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

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

Introduction

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

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

Method

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

A rectangular shape was used for the bending test (60x10x10 mm3) and for hardness test (20x10x10 mm3), a cubic geometry (10 mm 3) was selected for the compression test.

Mechanical tests

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

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

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

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

Its mechanical behavior can be distinguished into 3 phases:

- 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 ($\varepsilon_{max}(app)$).
- 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 (ε_{dens}) reached. The denser the material, the quicker the porosities are filled and the quicker the densification phase occurs.

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

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

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

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

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

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

Figure 3 : Shore A hardness test

Statistical analysis

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

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

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

Results

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

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

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

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

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

The values measured for the deformation εmax (app) associated with the maximum stress showed no difference at the same time (p = 0.949) for the 3 groups tested.

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

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

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

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

We note a clear correlation between variables "Young’s modulus" and "tensile strength" of the bending test (ρ evolving between 0.738 and 0.917) and a perfectly similar dependence (ρ ranging from 0.714 - 0.895) between the parameters "apparent modulus" and "maximum effective stress" of the compression test. Correlations tests result in a significant linear relationship between the Young's modulus and the stress at break in flexure on the one hand and the apparent modulus and maximum effective stress evaluated by compression test on the other hand.

Discussion

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

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

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

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

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

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

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

Conclusion

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

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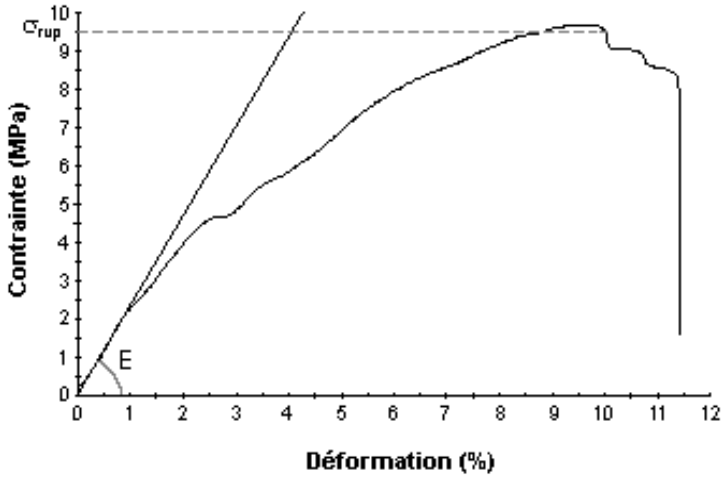

Figure 1: stress-strain curve of a fresh bone subjected to a 3-point bending test

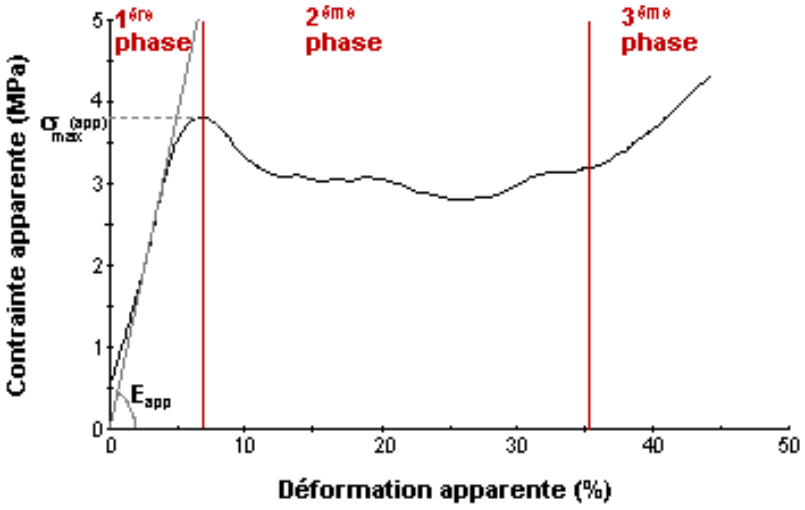

Figure 2: stress-strain curve of trabecular bone subjected to compression test

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

mechanical characterization	fresh bones	dry bones	Dry bones moistened	
3-point bending				
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

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

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

Os FRAIS	σ_{break}(app) (MPa)	ε_{break}(app) (%)	E (MPa)
σ_{break}(app) (MPa)	ρ = 1	ρ= 0.539 p-value: 0.054	ρ = 0.855 p-value<0.001
ε_{break}(app) (%)		ρ=1	ρ= 0.588 p-value: 0.037
E (MPa)			ρ=1

Os SEC	σ_{break}(app) (MPa)	ε_{break}(app) (%)	E (MPa)
σ_{break}(app) (MPa)	ρ = 1	ρ = 0.0.71 p-value: 0.433	ρ = 0.738 p-value: 0.018
ε_{break}(app) (%)		ρ = 1	ρ= - 0.595 p-value: 0.940
E (MPa)			ρ = 1

Os SEC réhumidifié	σ_{break}(app) (MPa)	ε_{break}(app) (%)	E (MPa)
σ_{break}(app) (MPa)	ρ = 1	ρ= - 0.050 p-value: 0.551	ρ = 0.917 p-value<0.001
ε_{break}(app) (%)		ρ = 1	ρ= - 0.417 p-value: 0.868
E (MPa)			ρ = 1

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

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

Os FRAIS	σ_{max}(app) (MPa)	ε_{max}(app) (%)	E_{app} (MPa)
σ_{max}(app) (MPa)	ρ = 1	ρ = 0.071 p-value: 0.433	ρ = 0.714 p-value: 0.023
ε_{max}(app) (%)		ρ=1	ρ= - 0.024 p-value: 0.522
E_{app} (MPa)			ρ=1

Os SEC	σ_{max}(app) (MPa)	ε_{max}(app) (%)	E_{app} (MPa)
σ_{max}(app) (MPa)	ρ = 1	ρ = 0.583 p-value: 0.05	ρ = 0.733 p-value: 0.012
ε_{max}(app) (%)		ρ = 1	ρ= 0.217 p-value: 0.288
E_{app} (MPa)			ρ = 1

Os SEC réhumidifié	σ_{max}(app) (MPa)	ε_{max}(app) (%)	E_{app} (MPa)
σ_{max}(app) (MPa)	ρ = 1	ρ= 0.077 p-value: 0.406	ρ = 0.895 p-value<0.001
ε_{max}(app) (%)		ρ = 1	ρ= - 0.245 p-value: 0.778
E_{app} (MPa)			ρ = 1