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ORIGINAL ARTICLE

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# Effect of a supercritical $CO_2$ based treatment on mechanical properties of human cancellous bone

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**Abstract** Bone allografts can be treated by various techniques before implantation. Recently, treatments based on supercritical carbon dioxide ($CO_2$) were developed. The goal of this study was to evaluate the influence of such a treatment on the biomechanical properties of bone allografts. Thirteen human femoral heads obtained from patients who had undergone hip arthroplasty were cut along the frontal plane yielding to two slices with similar mechanical properties. For each femoral head, one of the two slices (randomly chosen) was fresh-frozen, whereas the other one underwent all steps of a supercritical $CO_2$ based treatment in order to clean and secure bone tissue. Nine specimens (7 mm×9 mm×10 mm) per slice were then cut and loaded under compression in a physiologic saline solution maintained at 37°C. For the maximal compressive strength a mean value (SD) of 9.6 (2.4) MPa for fresh bone and 10.2 (5.2) MPa for treated one was found. Regarding the Young's modulus a mean value of 417 (85) MPa was obtained for fresh specimens and 412 (149) MPa for the treated ones. No statistical difference was found between the bone specimens treated with supercritical $CO_2$ and the fresh-frozen paired specimens when considering maximal compressive strength, Young's modulus and work to failure.

**Keywords** Supercritical fluids · Bone allograft · Femoral head · Cancellous bone · Biomechanical properties

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**Effet d'un traitement utilisant le $CO_2$ supercritique, sur les propriétés mécaniques de l'os spongieux humain**

**Résumé** Les allogreffes osseuses peuvent être traitées par différents procédés avant implantation. Récemment le dioxyde de carbone ($CO_2$) supercritique a été utilisé comme base d'un traitement. Le but de cette étude était d'évaluer l'influence d'un tel procédé sur les propriétés biomécaniques d'allogreffes osseuses. Treize têtes fémorales, provenant de patients ayant eu une arthroplastie totale de hanche, ont été découpées selon le plan frontal en deux tranches ayant des propriétés mécaniques similaires. Pour chaque tête fémorale, une des deux tranches (de façon aléatoire) était congelée fraîche, alors que l'autre subissait les différentes étapes du traitement utilisant le $CO_2$ supercritique. Neuf échantillons (7 mm×9 mm×10 mm) par tranche ont été découpés puis sollicités en compression dans un bain de sérum physiologique maintenu à 37°C. Pour la contrainte maximale en compression, la moyenne (écart type) est de 9.6 (2.4) MPa pour l'os frais et de 10.2 (5.2) MPa pour l'os traité. Quant au module d'Young les valeurs sont de 417 (85) MPa pour les échantillons frais et de 412 (149) MPa pour les échantillons traités. Aucune différence statistique, n'a été trouvée pour des échantillons traités par $CO_2$supercritique par rapport à des échantillons appariés, frais et congelés, lorsque l'on considère la contrainte maximale à la rupture, le module d'élasticité et l'énergie emmagasinée à la rupture.

**Mots-clés** Fluides supercritiques · Allogreffe osseuse · Tête fémorale · Propriétés biomécaniques · Os spongieux

## Introduction

The use of femoral cancellous bone is now a routine practice in orthopedic surgery. Sterilization processes were developed to increase bone graft safety and osteoconduction to facilitate the use of such bone allografts in the orthopedic surgery. They are generally an association of lipid extraction and protein oxidation. In classical processes, a liquid solvent is necessary (chloroform or acetone) to delipidate the bone tissue in order to improve osteoconduction [15]. The great quantity of lipids reduces the chemical action of the liquid oxidizing agents. Due to this limited diffusion of chemical agents into the bone porosity, the volume of the blocks treated with efficiency, is limited. In addition, the use of liquid solvents can leave in the tissue, undesirable toxic residues.

Supercritical fluids, especially supercritical carbon dioxide ($CO_2$), have been used for a long time in pharmaceutical and cosmetic industries for extraction, fractionation and chromatography. Using this technology to clean bone grafts is more recent. A first study on the use of supercritical $CO_2$ for bone lipid extraction was published in 1994 [6]. This study indicates high efficiency of this treatment for bone cleaning. In others studies, the same authors demonstrate the viro-inactivation potential of supercritical $CO_2$ [7] and the osteoconductive properties of bone grafts processed with this technology [8]. Beyond specific and critical temperature and pressure conditions, $CO_2$ is neither liquid nor gas. Its state is said to be supercritical. As is the case with liquids, it possesses high density and consequently, it has a greater "solvent" capacity. Supercritical $CO_2$ also displays another most interesting feature, namely that it has "transport" characteristics similar to those of gas because of low viscosity and high diffusion properties. As a result of such high diffusing and solubilizing properties, supercritical $CO_2$ is a method of choice to remove lipids contained in a porous matrix like that found in cancellous bones.

Bone allografts are widely used in orthopedic surgery for their mechanical strength. This essential property could be affected by the process applied as shown in several studies [4, 5, 11, 16].

The goal of this study was then to evaluate the influence of a supercritical $CO_2$ based treatment on the biomechanical properties of cancellous bone allografts from femoral heads.

## Materials and methods

Thirteen femoral heads obtained from patients who had undergone hip arthroplasty were selected for this study. The femoral heads were then cut along the frontal plane using a band saw (Type MKB 649 D, Murin-Fouillat, Lyon) to obtain two adjacent and symmetric [9] slices (15 mm). One underwent the supercritical $CO_2$ based treatment (called thereafter treated), whereas the other one was left untreated and was used as a control slice (called thereafter fresh) (Fig. 1). The treated slices were randomly chosen to prevent biased choices.

The control slice was frozen (−20°C) until the biomechanical testing and the other one went through all the supercritical $CO_2$ based treatment steps (Supercrit process, Biobank, Presles-en-Brie, France).

The Supercrit process is a method based on the lipid extraction of the bone tissue by a nontoxic fluid, the supercritical $CO_2$, with a chemical oxidation of the proteins remaining in the bone cavities.

Process steps :

1. Lipid extraction through supercritical $CO_2$ (26 MPa, 50°C)
2. Hydrogen peroxide 35% (2 h, 40°C)
3. Sodium hydroxide 4% (1 h)
4. Neutralization step using sodium dihydrogen phosphate 1.2% (30 min)
5. Ethanol 96% (3 h) and 99% (2 h)
6. Air-flow drying at 40°C (12 h)

Thanks to the efficiency of supercritical $CO_2$ in addition to hydrogen peroxide to remove fat, the lipid extraction rate is greater than 99.5% [6].

Each slice was then cut using a low speed diamond saw (Isomet 2000, Buehler) into 9 parallelepiped (7 mm×9 mm×10 mm) (Fig. 2). These different dimensions allow the different anatomic directions to be defined.

Parallelepiped specimens were then submitted to a destructive compressive test using a testing machine

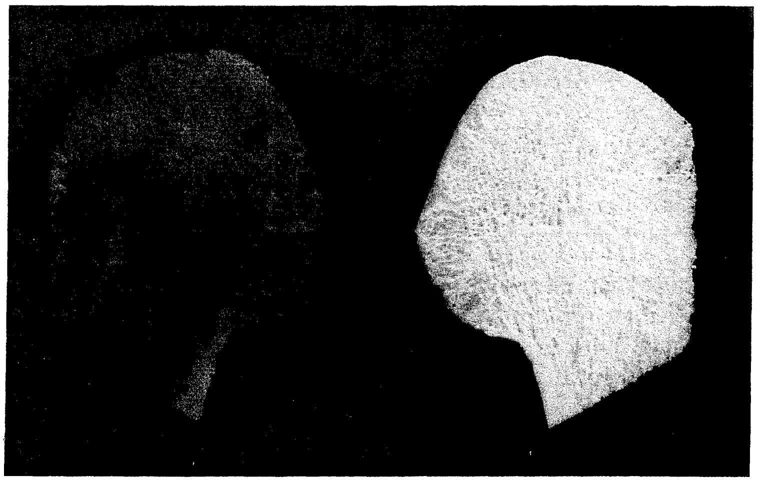


**Fig. 1** Symmetry along the frontal plane of the fresh part of the femoral head (*left*) and treated part (*right*)

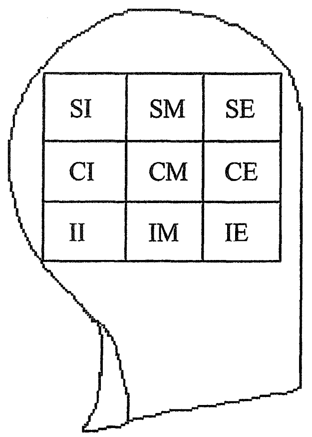



**Fig. 2** Location of the 9 specimens in the femoral head. SI: supero-internal, SM: supero-medial, SE: supero-external, CI: central-internal, CM: central-medial, CE: central-external, II: infero-internal, IM: infero-medial, IE: infero-external

(Instron 5500R) in a physiologic saline solution maintained at 37°C [13]. Tests were conducted along the infero-superior axis which corresponds to the primary compressive group in the femoral head.

The compressive test was performed at low speed $10^{-3}$ $s^{-1}$ = 5 mm $min^{-1}$ which is below 10 $s^{-1}$ the limit found by Carter and Hayes [3] to avoid viscoelastic effect due to bone marrow.

The compliance of the system was corrected to have a true measurement of the bone displacement. In order to improve the reproducibility of the compressive test, a 10 cycle-precycling (up to 0.8%) was first applied to the specimens [10].

From the strength-strain curves the Young's modulus, the maximum strength and the energy to failure were computed.

To evaluate the influence of the treatment and of the specimen location in the femoral head, a repeated measures 2-way analysis of variance was performed using the software R (R Foundation for Statistical Computing, Vienna, Austria).

## Results

Tests were finally successful for 103 fresh specimens and 99 treated ones (extracted from 13 femoral heads). Maximal strengths are given for fresh specimens (Table 1) and treated ones (Table 2).

As shown in Tables 1 and 2, it was often impossible to cut specimens from locations superior-medial (SM) and inferior-internal (II) due to cancellous bone insufficiency or femoral head being too small in size.

A mean value (SD) of 10.2 (5.2) MPa for treated bone and 9.6 (2.4) MPa for fresh one was found.

Young's moduli are given for fresh specimens (Table 3) and treated specimens (Table 4). A mean value of 417 (85) MPa was found for fresh specimens and 412 (149) MPa for the treated ones.

High correlations were found between maximal compressive strength and Young's modulus. These regressions gave a slope of 29.22, $R^2=0.95$ and a slope of 31.44, $R^2=0.81$ respectively for fresh and treated specimens (Fig. 3).

For the work to failure, a mean value of 182 (58) KJ $m^{-3}$ was found for fresh specimens and 241 (104) KJ $m^{-3}$ for the treated ones.

To perform the statistical analysis using a repeated measures 2-way analysis of variance, a subset of the previous results were considered (39 specimens for the strength and the Young's modulus, 26 for the work to failure) since, the column with missing data had to be removed for this statistical analysis. No statistical difference due to the supercritical $CO_2$ based treatment was found either for the maximal compressive strength ($P=0.20$), or the Young's modulus ($P=0.25$), nor the work to failure ($P=0.25$). The location of the specimens in the femoral head also had no statistical difference on the biomechanical parameters.

**Table 1** Maximal compressive strength for fresh cancellous bone specimens ($n=103$)

| Head by Location | SE | SM | SI | CE | CM | CI | IE | IM | II | Mean by head (SD) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 9.7 | – | 8.7 | – | 9.6 | 3.6 | 6.1 | – | 10.2 | 8.0 |
| 2 | 10.1 | 15.8 | 9.8 | 11.3 | 18.0 | 9.9 | 10.8 | 17.7 | 9.8 | 12.6 |
| 3 | 4.8 | 16.8 | 20.7 | 11.4 | 20.7 | 12.6 | 12.2 | 19.0 | 6.8 | 13.9 |
| 4 | 5.8 | – | 14.2 | 6.9 | 14.9 | 7.7 | 6.6 | 14.1 | 12.9 | 10.4 |
| 5 | 8.7 | – | 8.2 | 6.4 | 13.2 | 9.3 | 5.7 | 13.1 | 9.2 | 9.2 |
| 6 | 6.7 | – | 11.4 | 9.6 | 15.5 | 6.3 | 7.7 | 14.0 | 4.1 | 9.4 |
| 7 | 6.5 | – | 5.7 | 3.5 | 7.6 | 4.6 | 4.5 | 8.3 | 4.1 | 5.6 |
| 8 | 10.9 | – | – | 8.4 | 15.5 | 14.7 | 9.8 | 13.8 | 7.5 | 11.5 |
| 9 | 8.6 | – | 7.1 | 7.5 | 10.3 | 5.3 | 8.1 | 11.6 | 4.9 | 7.9 |
| 10 | 8.6 | 12.6 | – | 7.5 | 12.8 | 7.0 | 6.3 | 9.2 | 5.8 | 8.7 |
| 11 | – | 14.1 | 6.7 | 11.0 | 16.0 | 6.9 | 10.3 | 17.5 | – | 11.8 |
| 12 | 6.7 | 19.1 | 8.1 | 7.2 | 13.8 | 5.9 | 5.9 | 13.3 | 6.9 | 9.7 |
| 13 | 8.7 | 8.0 | – | 8.0 | 7.9 | 2.9 | 7.1 | 8.1 | 2.8 | 6.7 |
| Mean by location | 8.0 | 14.4 | 10.0 | 8.2 | 13.5 | 7.5 | 7.8 | 13.3 | 7.1 | 9.6 (2.4) |

**Table 2** Maximal compressive strength for treated cancellous bone specimens ($n = 99$)

| Head by Location | SE | SM | SI | CE | CM | CI | IE | IM | II | Mean by head (SD) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.1 | – | 5.2 | 5.6 | 5.1 | 2.9 | 5.1 | – | 5.2 | 5.0 |
| 2 | 18.3 | – | 9.0 | 5.4 | 15.9 | 6.6 | 6.9 | 14.2 | 4.7 | 10.1 |
| 3 | 4.4 | 22.2 | 11.9 | 7.7 | 20.3 | 10.2 | 16.2 | 8.6 | 25.1 | 14.1 |
| 4 | – | 26.3 | – | 4.8 | 18.2 | 7.8 | 4.2 | 20.6 | 12.2 | 13.4 |
| 5 | 9.1 | – | 12.7 | 7.2 | 21.5 | 12.3 | 12.1 | 32.4 | 12.8 | 15.0 |
| 6 | 17.6 | 29.7 | 13.7 | 23.6 | 32.5 | 13.2 | 27.0 | – | 27.0 | 23.0 |
| 7 | 4.2 | – | 5.9 | 3.2 | 5.6 | 4.0 | 3.3 | 5.8 | 2.6 | 4.3 |
| 8 | 8.4 | – | 13.0 | 9.2 | 14.0 | 5.3 | 7.9 | 13.6 | 4.5 | 9.5 |
| 9 | 5.8 | – | 13.7 | 3.5 | 9.8 | 5.5 | 1.5 | 5.5 | 2.2 | 5.9 |
| 10 | 3.9 | – | 7.6 | 5.5 | 11.2 | 6.7 | 7.7 | 10.4 | – | 7.6 |
| 11 | – | 13.4 | 4.8 | 9.4 | 14.4 | 5.0 | 11.7 | 9.8 | – | 9.8 |
| 12 | 8.8 | 9.7 | 11.9 | 5.6 | 10.2 | 4.2 | 7.4 | 9.8 | – | 8.4 |
| 13 | 5.5 | – | – | 4.4 | 9.6 | 2.9 | 7.2 | 7.1 | – | 6.1 |
| Mean by location | 8.4 | 20.2 | 9.9 | 7.3 | 14.5 | 6.7 | 9.1 | 12.5 | 10.7 | 10.2 (5.2) |

**Table 3** Young's modulus for fresh cancellous bone specimens ($n = 103$)

| Head by Location | SE | SM | SI | CE | CM | CI | IE | IM | II | Mean by head (SD) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 371 | – | 277 | – | 392 | 138 | 285 | – | 407 | 312 |
| 2 | 401 | 597 | 380 | 447 | 685 | 433 | 473 | 665 | 427 | 501 |
| 3 | 203 | 560 | 659 | 442 | 718 | 477 | 422 | 624 | 294 | 489 |
| 4 | 269 | – | 528 | 335 | 565 | 328 | 282 | 532 | 516 | 419 |
| 5 | 302 | – | 318 | 257 | 509 | 404 | 205 | 504 | 326 | 353 |
| 6 | 272 | – | 449 | 424 | 588 | 260 | 347 | 546 | 141 | 378 |
| 7 | 370 | – | 385 | 222 | 476 | 326 | 299 | 513 | 281 | 359 |
| 8 | 542 | – | – | 495 | 750 | 689 | 570 | 716 | 439 | 600 |
| 9 | 460 | – | 424 | 425 | 569 | 309 | 474 | 649 | 304 | 452 |
| 10 | 384 | 515 | – | 356 | 521 | 290 | 299 | 414 | 254 | 379 |
| 11 | – | 525 | 294 | 452 | 612 | 320 | 464 | 617 | – | 469 |
| 12 | 279 | 698 | 396 | 339 | 600 | 296 | 261 | 550 | 312 | 415 |
| 13 | 348 | 351 | – | 341 | 347 | 141 | 301 | 364 | 141 | 292 |
| Mean by location | 350 | 541 | 411 | 378 | 564 | 339 | 360 | 558 | 320 | 417 (85) |

**Table 4** Young's modulus for treated cancellous bone specimens ($n = 99$)

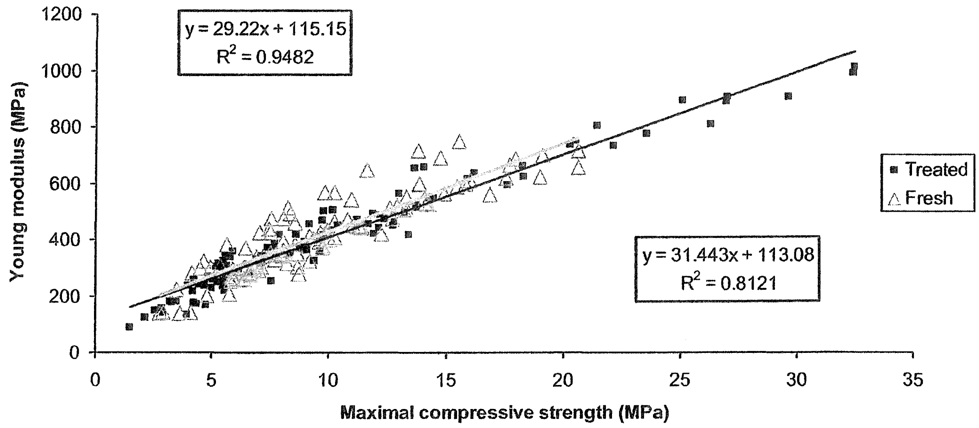
| Head by Location | SE | SM | SI | CE | CM | CI | IE | IM | II | Mean by head (SD) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 294 | – | 248 | 305 | 265 | 155 | 257 | – | 305 | 262 |
| 2 | 623 | – | 394 | 299 | 615 | 313 | 330 | 546 | 234 | 419 |
| 3 | 241 | 733 | 422 | 373 | 738 | 436 | 634 | 415 | 894 | 543 |
| 4 | – | 810 | – | 269 | 662 | 352 | 216 | 748 | 488 | 506 |
| 5 | 363 | – | 449 | 342 | 804 | 470 | 440 | 987 | 465 | 540 |
| 6 | 594 | 907 | 521 | 776 | 1010 | 499 | 904 | – | 889 | 762 |
| 7 | 257 | – | 359 | 180 | 343 | 237 | 178 | 337 | 146 | 255 |
| 8 | 350 | – | 564 | 455 | 658 | 315 | 418 | 654 | 242 | 457 |
| 9 | 279 | – | 515 | 182 | 499 | 252 | 89 | 325 | 121 | 283 |
| 10 | 135 | – | 251 | 235 | 473 | 277 | 386 | 450 | – | 315 |
| 11 | – | 418 | 168 | 324 | 551 | 228 | 453 | 415 | – | 365 |
| 12 | 372 | 359 | 494 | 273 | 505 | 178 | 371 | 469 | – | 378 |
| 13 | 220 | – | – | 172 | 410 | 137 | 314 | 343 | – | 266 |
| Mean by location | 339 | 645 | 399 | 322 | 580 | 296 | 384 | 517 | 420 | 412 (149) |

## Discussion

This study evaluated the treatment influence on the biomechanical properties of cancellous bone specimens extracted from femoral head. The biomechanical evaluation was based on the maximal compressive strength, the Young's modulus and the work to failure.

Due to interindividual variations in biomechanical properties, comparisons were made using paired specimens in the same femoral head. The frontal plane was chosen as a symmetry plane taking into account the mechanical property distributions in the cancellous bone of the femoral head [2, 9]. Using this cutting protocol symmetry of the central slices seems reliable. Moreover

Fig. 3 Correlation between the maximal compressive strength and the Young's modulus for fresh (*grey line*) and treated specimens (*black line*)




the treated slices were randomly chosen to prevent biased choices.

The biomechanical parameters were compared to those of previous studies. The maximal compressive strength is in the range of the results of previous studies (Table 5). And Young's modulus (mean and (SD)) values (417 MPa (85) for fresh bone), were similar to those found by Schoenfeld et al. [14] (345 MPa) and Brown and Ferguson [2] (340 (102) MPa). The value of 182 (58) KJ $m^{-3}$ for work to failure is also in the range of the results obtained by Cornu et al. [5]. These similar results on fresh specimens give confidence in the protocol used for the evaluation of biomechanical properties. The comparisons must be made considering the differences in terms of subjects (age, pathologies...) and biomechanical testing protocols. However the goal in the current study was to evaluate the influence of a treatment on the biomechanical properties of cancellous bone specimens using the same testing protocol.

A higher standard deviation was found for the mechanical properties in the treated group in comparison to the control/fresh one. This difference was mainly due to the specimen 6 in the treated group. However no reasons can be given to reject this femoral head. Moreover the data analysis was performed without the femoral head number 6 and the conclusions on the effect of the treatment were the same.

To evaluate the influence of the treatment, the comparison was based on fresh-frozen specimens (control) and treated ones. The sterilization process was not considered in the treatment since it is mandatory and

**Table 5** Maximal compressive strength from cancellous bone specimens tested along the primary compressive group

| Studies | Number | Age | Strength: mean (SD) MPa | Min–Max MPa |
|---|---|---|---|---|
| [1] | 12 | 73 | 5.8 | 1.4–11.7 |
| [12] | 6 | 45 | 9.3(4.5) | |
| Current study | 13 | 73.5 | Treated: 10.2 (5.2) | 5.6–13.9 |
| | | | Fresh: 9.6 (2.4) | 4.3–23 |

whatever may be the conservation mode of bone graft. Moreover according to Vastel et al. [16], gamma irradiation at doses used in orthopedic practice (about 25 kGy) only slightly reduced (around 2%) mechanical properties of cancellous bone specimens.

Regarding the influence of the supercritical $CO_2$ based treatment, no statistical difference was found in the current study for each biomechanical parameter. This result is in agreement with the results given by Fages et al. [6] on a limited number of specimens and less biomechanical parameters. The brittleness of the allografts (evaluated by the work to failure) was not affected by the supercritical $CO_2$ based treatment. A similar result was previously observed on freeze-dried specimens (without gamma irradiation) [5].

With or without treatment there was a same distribution of the mechanical properties in the femoral head. Maximum values were found in the central zone (medial, Tables 1, 2, 3, 4) which is in accordance with the location of the primary compressive group of the femoral head.

The effect of supercritical based treatment could be compared to other bone treatments, in particular to that of Cornu et al. [5]. They have shown that water jet washing and immersion in a chloroform/ethanol solution reduced the maximal compressive strength and the Young's modulus by 18.9% and 20.2%, respectively. In a treatment including acetone and 6 M urea, Vastel et al. [16] showed a loss of 34% of the failure strength in the treated bone compared to the fresh-frozen bone.

The results of the current study could be based on the way of action of supercritical $CO_2$. Lipid extraction is performed using a supercritical $CO_2$ flow which is nontoxic for collagenic protein responsible of natural bone rigidity [6]. The $CO_2$ flow acts indepth by collecting the lipids contained in the pores of the trabecular network with the help of a very low viscosity. This property avoids the use of a pressurized water jet to eliminate the lipids dissolved by an organic solvent like chloroform or acetone [5]. Moreover, the diffusion of the secondarily-used oxidizing chemical agents (hydrogen peroxide, sodium hydroxide) is facilitated, which makes it possible to decrease the processing times and probably to minimize an excessive denaturation of bone tissue.

In the current study the specimens are from elderly subjects (73.5 years old in average) who had undergone hip arthroplasty. In these conditions the treatment based on supercritical $CO_2$ induces no modification of the mechanical properties of the bone allograft. This is a very important point since, bone in a tissue bank comes in majority from hip arthroplasty [16].

Finally, the results confirmed the high correlation between maximal compressive strength and Young's modulus even in the treated specimens [12]. Such a correlation could be used to assess the mechanical properties of cancellous bone allograft. For example, a nondestructive assessment such as the ultrasonic measurement [16] could be performed before furnishing the bone allograft to the surgeon.

## Conclusion

This study showed that a treatment based on supercritical $CO_2$ does not modify the biomechanical properties of cancellous bone, either the maximal compressive strength ($P=0.20$), or the Young's modulus ($P=0.25$), or the work to failure ($P=0.25$).

The confirmed high correlation between the maximal compressive strength and the Young's modulus enable to consider the single evaluation of the Young's modulus to assess nondestructively the maximal compressive strength of cancellous bone allograft.

Finally, human cancellous bone treated with a supercritical $CO_2$ based process has the same compressive behavior as fresh specimens from a biomechanical point of view.

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