José Luis Díaz León, et al., Int. J. of Design & Nature and Ecodynamics. Vol. 11, No. 4 (2016) 722–730

MECHANICAL CHARACTERIZATION OF BONE ALLOGRAFTS, ENRICHED WITH MESENCHYMAL CELLS

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ABSTRACT

Bone allografts, which are used as bone regeneration material, must satisfy two functions: a biologic function and a mechanical function. The biologic task is satisfied by enrichment of the osseous reconstructive material with mesenchymal cells, radio-sterilized and lyophilized, which helps to a more efficient formation of new bone. Regarding the mechanical function, the allografts must be as rigid and strong as intact bone for immediate load-bearing capability. Consequently, a good mechanical characterization is needed to guarantee the structural integrity of the allografts in the host tissue. Thus, in this work results are presented from compression testing of cancellous and cortical/cancellous allograft tissue chips, as well as results on flexure and pure shear testing of cortical allograft strips. The test specimens were fabricated according to standard procedures. For the cancellous graft chips, different elastic moduli were obtained a long the three perpendicular directions, 32.2, 98.2, and 162.4 MPa, showing orthotropic behavior. For the cortical/cancellous chips, compression loads were only applied along the longitudinal and transversal directions of the cortical phase; the corresponding elastic moduli displayed were 518.6 and 384.8 MPa. On the other hand, for the cortical graft strips, the flexure elastic modulus obtained was 38.9 GPa; reported flexure elastic modulus in the literature for fresh human bone are between 1.525 and 31.5 GPa. Finally the shear strength exhibited by the cortical graft was 43.6 MPa.
Keywords: biomechanics, complex material, mechanical bone, tissue engineering.

1 INTRODUCTION

Bones are rigid organs that consist of osseous tissue, bone marrow, endosteum, periosteum, cartilage, nerves, and vascular channels. Osseous tissue, which realizes mechanical functions, is formed by connective tissue cells in an extracellular matrix composed mainly of minerals, proteins, and water. The bone composition and configuration will vary according to factors such as the anatomical location, supported load, age and gender of the person, and the possible diseases that such individual could suffer. In regard to bone composition, mineral phase comprises about 65 wt.% and water around 10 wt.%, whereas the rest part is an organic matrix of collagen. According to its structure, osseous tissue may be cancellous (trabecular) or cortical (lamellar). Cancellous bone is a network of interconnected porosities, ranging between 50% to 90% of empty space, with a solid portion that is formed of struts and plates that can adopt different configurations. It is located at the epiphysis of long bones and the interior of cuboid bones. Cortical tissue is located at the bone surface, and it has a homogeneous and compact macrostructure. It is found mainly at the bone diaphysis and its thickness varies according to the bone anatomical location [1].

Bones have mechanical, synthetic, and metabolic functions. The mechanical functions are protection of internal organs, body support, and interaction with muscles and tendons to generate body movement. The synthetic function is conducted by the bone marrow, where both bone and blood

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This paper is part of the Proceedings of the New Forest Conference on Complex Systems 2016 (Complex Systems 2016)
www.witconferences.com

ISSN: 1755-7437 (paper format), ISSN: 1755-7445 (online), http://www.witpress.com/journals
DOI: 10.2495/DNE-V11-N4-722-730

cells are synthesized. Metabolic functions are related to act as a reservoir of calcium, phosphorus, growth factors and fat.

Regarding the mechanical function, bones are the structural elements of the human body. Skeletal system supports loads due to the different activities of an individual as holding things, walking, running, and so forth. These loads induce tensile, compressive, or shear stresses on the bone tissue. More complex stress states, such as those caused by bending or twisting of bone, can be decomposed into the three basic above-mentioned stresses. To study these stresses, bone mechanical properties such as elasticity modulus, compressive, and tensile strength are important. These properties are highly dependent on the position of the bone and the condition of the individual. Besides, mechanical properties of bone vary depending on the load orientation with respect to the orientation of the tissue (anisotropy) and the speed to which the load is applied (viscoelasticity). Some important mechanical properties are described in Table 1.

Bone tissue as an engineering material can fail because mechanical loads originate stresses over the limits a healthy bone can bear or because the mechanical properties of bone are decreased by various pathologies making the bone weak and prone to be damaged. Usually, small bone defects heal spontaneously but large defects cannot regenerate without intervention. There are several options for the reconstruction of large bone defects that include bone grafts (autograft, allograft and xenograft) as well as bone constructs created by bone tissue engineering principles [2].

Autografts are osseous tissue taken from the same individual and are considered the "gold standard" because they are osteoinductive, osteoconductive, and osteogenic. This material is normally taken from a site that is not under mechanical load such as the iliac crest. Autografts contain cells and growth factors that support the process of bone regeneration and do not exhibit risk of rejection and disease transmission. Some drawbacks of autografts are the necessity of additional surgeries, possible infections, morbidity of the bone, pain, and its limited availability. Depending on the source of the osseous tissue there may also be allografts (tissue from individuals of the same species) or xenografts (tissue from individual of different species, animals in particular). Xenografts, could be osteoinductive and osteoconductive and with low cost with high availability but have the disadvantages of immune response and risk of transmission of animal diseases.

Allografts presented benefits as ready availability and easy handling but require treatments such as freeze drying, irradiation, and washing with acid, among others, to prevent rejection by the receptor and remove any possible infections from the tissue to be implanted; these processes can affect their mechanical and biological properties. Thus, allografts retain osteoconductivity and

Table 1: Property of bone.

Property	Cortical bone	Cancellous bone
Tensile strength (MPa)	50–150	10–100
Compressive strength (MPa)	130–230	2–12
Young's modulus (GPa)	7–30	0.02–0.5
Strain to failure (%)	1–3	5–7
Shear strength (MPa)	53–70	
Shear modulus (GPa)	3	

osteoinductivity but they do not possess osteogenic cellular components [3]. This lack of osteogenic cells leads to the creation of allogenic bone grafts added with mesenchymal stem cells (MSCs), from bone marrow aspirates, to provide all three aspects of bone healing. Then, it remains to know how the mechanical properties of the allografts enhanced with MSCs are affected. Consequently, in this work, results are pre- sented from compression testing of allograft tissue chips, as well as results on flexure and pure shear testing of cortical allograft strips.

2 MATERIALS AND METHODS

Human bones are subjected to different loading conditions: tensile, compression, flexure, shear, torsion and combinations of all of them. Fractures produced by tensile loading generally appear in bones with a considerable portion of cancellous osseous tissue. Fractures due to compression loading frequently emerge in vertebras and in some articulations of elderly individuals. Shear failures are present in almost all bones, mainly those where torsion or combined loads are present. Flexure fractures become visible in long bones where the uniform stress distribution is altered due to unsymmetrical cross sections distinctive in such bones [4, 5].

The bone allografts characterized in the present work, which derive from long and flat human-donated bones, are used for filling of bone defects, prosthetic or bone graft augmentation for general orthopaedic reconstruction [6, 7]. Once implanted, the grafts will be under a combination of tension, compression and shear stresses [8]. Thus, the mechanical behavior of the bone allografts is experimentally studied by performing compression, shear, and bending tests to get their corresponding elastic and strength properties. It is worth noting that the mechanical properties obtained under dry conditions are different from those obtained in hydrated bones (Fig. 1a), which occurs when the bone grafts are being osteo-integrated after implantation [910–11].

The raw material to be tested consisted of cancellous and cortical/cancellous allograft chips (average size 10 mm), as well as small cortical allograft strips. Thus, particular fixtures and specimens were designed according to standard test procedures. In addition, because the mechanical response of bone is loading rate dependent, Fig. 1b [12], the different tests were performed at a travelling head speed of 1 mm/min in the testing machines, which corresponds to the lower limit of the recommended speed range (1–5 mm/min) established in the standards [13, 14, 15].

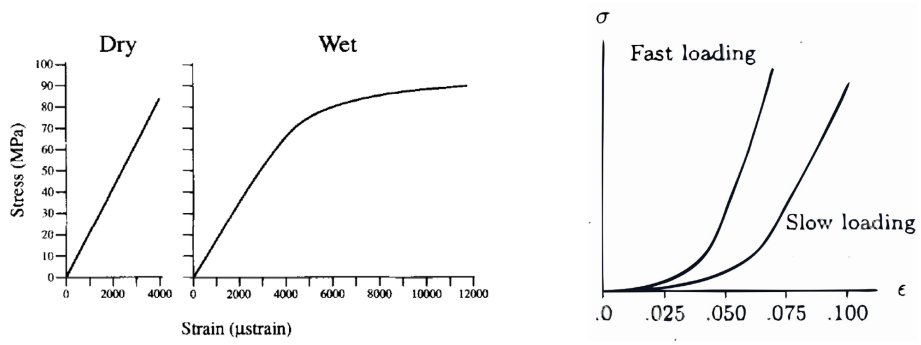

Figure 1: Graphics. (a) Stress-strain behavior of dry and hydrated bone. (b) Effect of loading rate on stress-strain behavior.

2.1 Compression testing

Several researchers have studied the orthotropic behavior of bone by testing full and partial specimens conformed by cancellous, cortical or cancellous/cortical osseous tissue [12, 16]. Fantner *et al.* [17] tested cancellous chips of 5 × 5 × 4 mm in compression. In the present investigation, cubic chips of 5 × 5 × 5 mm were carefully prepared from irregular-shaped cancellous allograft chips added with MSCs. These specimens were tested in compression along the three perpendicular directions to study their orthotropic behavior (Fig. 2a) [18]. In addition, cubic chips of 5 × 5 × 5 mm were also prepared from cancellous/cortical allograft material; however, in this case compression testing was done along the longitudinal and transversal directions, respect to cortical fibers direction (Fig. 2b) [19]. All tests were performed according to ASTM D1621 standard [13], in a Instron 1011 testing machine.

Turner and Burr [9] proposed a modified compression test fixture to circumvent possible misalignment respect to the loading axis and thus prevent bending effects (Fig. 3a). This misalignment can be due to non-parallel specimen loading faces or possible premature failure of struts or plates of the cancellous structure. The designed fixture consisted of replacing one of the compression plates for an articulated plate, which is free to rotate around the three perpendicular axes such that no bending moments are generated on the loading faces (Fig. 3b).

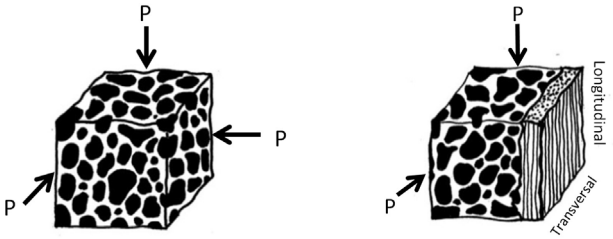

Figure 2: (a) Cancellous tissue chip. (b) Cancellous/cortical chip.

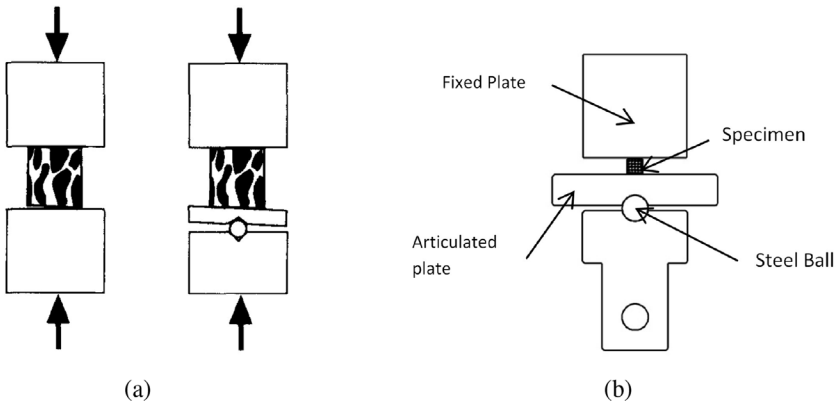

Figure 3: (a) Compression fixture, Turner and Burr [9]. (b) Designed fixture.

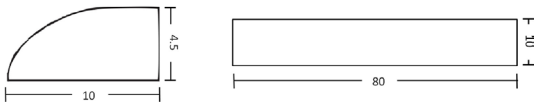

Figure 4: Bending specimen, dimensions in mm.

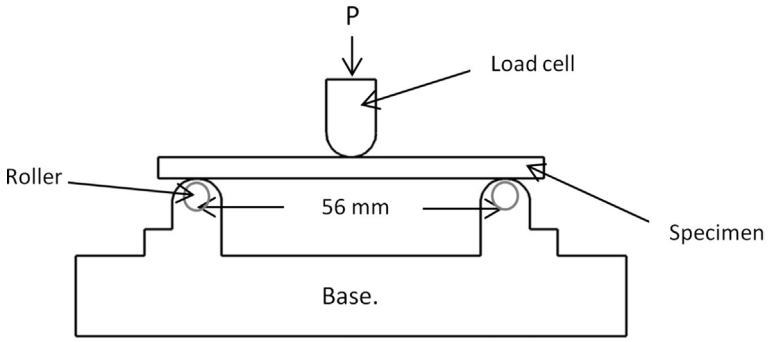

Figure 5: Three point bending fixture.

2.2 Flexure testing

Draper and Goodship [20] used four-point bending for flexure testing of hydrated cortical bone samples 10 mm width, 5 mm thickness, and 45 mm length. In the present case, three point bending tests were performed according to ISO 178 standard [14] on lyophilized cortical bone strips. The samples were cut along the bone fiber direction and the dimensions of the bending specimens are 80 mm length, 4.5 mm thickness, 10 mm width (Fig. 4). It is important to mention that the cross sectional area resembles an elliptical segment, which was maintained during the testing to fulfill the thickness/width aspect ratio established in the standard (Fig. 4). The three-point bending test arrangement is shown in Fig. 5, where the distance between supports (rollers) is 56 mm.

2.3 Shear testing

Shear tests were performed in accordance with the ASTM 5379 standard [15], which is based on the Iosipescu method. Due to material availability, the specimen size was reduced to one quarter of the standard size, following the guidelines for resizing established in the ASTM standard. One of the most important criteria to fulfill is a minimum thickness of 2.5 mm, although some favorable results have been reported when using 0.5 mm thickness [21]. Shear specimen length must be four times the width, with a 90 degree angle length centered notch. The notch depth is 20% of specimen width [9, 21]. The final shear specimen size was 20 mm length, 4 mm thickness, 5 mm width (Fig. 6).

As mentioned above, the Iosipescu method was used for shear testing. The Iosipescu fixture size was reduced to one quarter the size proposed in ASTM D5379 standard [15]. Stiffness of the fixture was a concern, as well as the alignment of the lower and upper grips with the specimen to provide pure shear loading (Fig. 7).

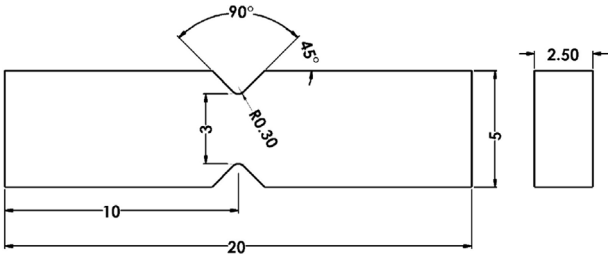

Figure 6: Shear specimen, dimensions in mm.

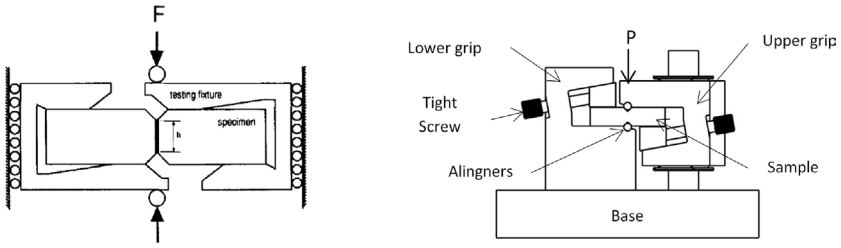

Figure 7: (a) Iosipescu fixture, Turner and Burr [9], (b) Designed fixture.

3 RESULTS

Elastic compression moduli along the three orthogonal directions were obtained from compression testing cancellous allograft chips. Figure 8a shows the initial linear part of stress–strain plots for the three loading directions and chip 8b. Each line represents the average of 5 tests, and the corresponding moduli obtained are 162.4 MPa, 98.2 MPa, and 32.2 MPa. Fig. 8c displays initial stress–strain plots for cancellous/cortical allograft chips tested in compression. Load was applied along the longitudinal (cortical fiber) and transverse (perpendicular to cortical fiber 8.d) direction. The moduli obtained were 518.6 MPa and 384.6 MPa, respectively.

Shear stiffness modulus was obtained from shear testing allograft cortical strips. Stress–strain plot shown in Fig. 9 represents the average of five tests. The average shear stiffness modulus obtained was 132.14 MPa.

Considering flexure testing of allograft cortical specimens, three tests were performed and Fig. 10a, shows the average stress-strain plot and shear specimen tested 10.b. From such plot, yield stress in bending was 204.15 MPa while the flexural elastic modulus was 24.411 GPa.

4 CONCLUSIONS

Mechanical properties were obtained from compression testing of cancellous and cortical/cancellous allograft chips, and from flexure and pure shear testing of cortical allograft strips. Design of scaled fixtures and specimens was crucial to carry out the mechanical characterization, due to restrictions in the availability of sampling materials as well as to specimen size that can be obtained from different bones. Orthotropic behavior was observed for the cancellous and cortical/cancellous allograft chips.

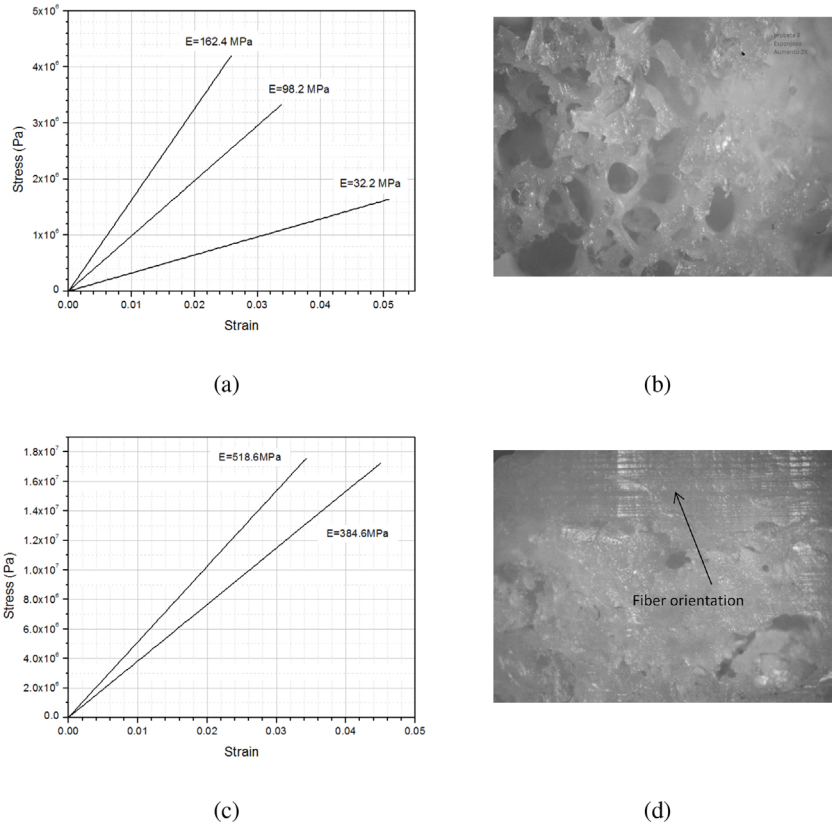

Figure 8: Stress–strain behavior. (a) Compression in cancellous chips along three orthogonal directions, (b) cancellous chip mcirostructure, (c) compression in cancellous/cortical chips along two orthogonal directions, (d) cancellous/cortical microstructure.

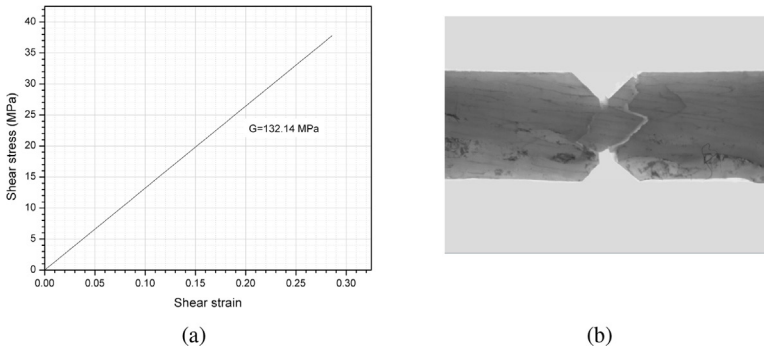

Figure 9: (a) Shear stress–strain behavior in cortical strips, (b) fractured specimen.

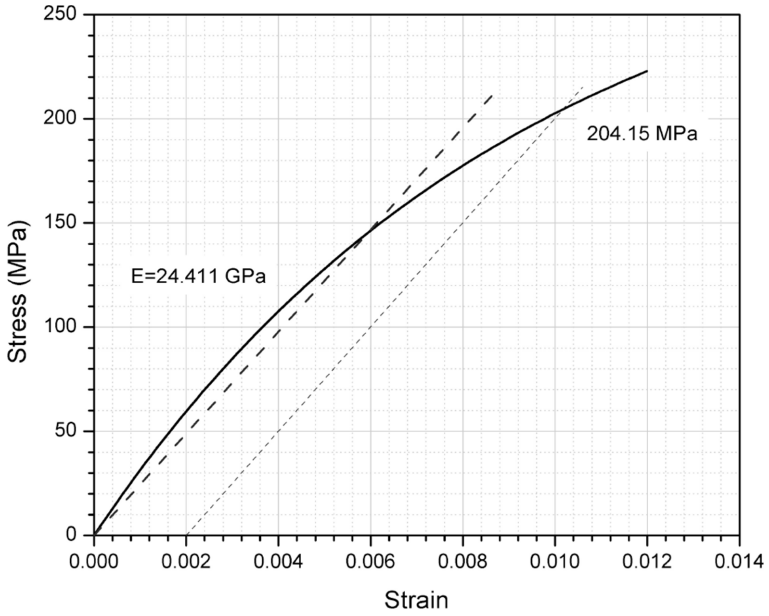

Figure 10: Bending stress–strain behavior in cortical strips.

By comparing the elastic moduli obtained in the present study with those reported in the literature (Table 1), it is observed that elastic moduli values (162.4 MPa, 98.2 MPa, and 32.2 MPa) for the cancellous allograft bones added with MSCs are in the range reported in the literature (20–500 MPa). On the other hand, elastic moduli values obtained for cortical/cancellous allografts added with MSCs (518.6 MPa and 384.6 MPa) are well below of those reported in the literature (7–30 GPa). Likewise, shear stiffness modulus (132.14 MPa) obtained in the present work is very low compared to the corresponding modulus reported (3 GPa). Finally, flexure elastic modulus (24.41 GPa) for cortical strips fall in the range reported in the literature.

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