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Links between mechanical behavior of cancellous bone and its microstructural properties under dynamic loading

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ABSTRACT

Previous studies show that *in vivo* assessment of fracture risk can be achieved by identifying the relationships between microarchitecture description from clinical imaging and mechanical properties. This study demonstrates that results obtained at low strain rates can be extrapolated to loadings with an order of magnitude similar to trauma such as car crashes. Cancellous bovine bone specimens were compressed under dynamic loadings (with and without confinement) and the mechanical response properties were identified, such as Young's modulus, ultimate stress, ultimate strain, and ultimate strain energy. Specimens were previously scanned with pQCT, and architectural and structural microstructure properties were identified, such as parameters of geometry, topology, connectivity and anisotropy. The usefulness of micro-architecture description studied was in agreement with statistics laws. Finally, the differences between dynamic confined and non-confined tests were assessed by the bone marrow influence and the cancellous bone response to different boundary conditions. Results indicate that architectural parameters, such as the bone volume fraction (BV/TV), are as strong determinants of mechanical response parameters as ultimate stress at high strain rates (p-value < 0.001). This study reveals that cancellous bone response at high strain rates, under different boundary conditions, can be predicted from the architectural parameters, and that these relations with mechanical properties can be used to make fracture risk prediction at a determined magnitude.

1. Introduction

Cancellous bone has a porous structure that protects the marrow contained in the cortical bone layer. A better understanding of bone fracture is necessary to improve the musculoskeletal modeling of the human body intended to aid in the design of protective features. The mechanical properties of cancellous bone, over a large range of strain rates, have been studied to better simulate and understand injuries sustained such as during a car crash (Chaary et al., 2007). Researchers have made great progress in characterizing the compressive behavior on cases of quasi-static loading (Guedes et al., 2006), and recently accounting for the influence of bone marrow (Halgrin et al., 2012; Charlebois et al., 2008). The few experimental studies entailing mechanical characterization at high strain rates have proved that the mechanical behavior is strain rate dependent (Linde et al., 1991). Furthermore, as cancellous bone is highly heterogeneous with a variation depending, among others, on age (Follet et al., 2011), anatomic localization (Morgan and Keaveny, 2001), and geometry (Linde et al., 1992), studies have recently demonstrated that the characterization of cancellous bone architecture was a good predictor of low strain rate mechanical response (Zhou et al., 2014). Given the daily life loadings, it would be interesting to characterize the dynamic response of cancellous bone in regards to its architectural description. However, there is a lack of this in the literature.

The aim of this study is to quantify the links between architectural descriptors of cancellous bone and macroscopic mechanical behavior under high strain rate compression loading for two boundary conditions.

2. Materials and Methods

2.1. Samples

Twenty-four distal segments of 3 years old bovine femoral bones were used for this study (72 h post-mortem) and frozen at −20 °C (Linde and Sørensen, 1993). Experiments were conducted within the animal welfare regulations and guidelines for the country. A diamond slitting wheel was used to isolate two cylinders of cancellous bone from each frozen bone in the sagittal plane (diameter 41 mm and thickness 14 mm). Specimens were then preserved in vacuum-sealed plastic bags; they were slowly thawed for 12 h at +5 °C before being exposed to room temperature (approximately +24 °C) prior to mechanical testing (Mitton et al., 1997).

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2.2. Microstructure properties

The peripheral quantitative tomodensitometry technique (pQCT) was used to identify the microstructure properties of each frozen cylinder (XtremeCT, Scanco Medical, cubic voxel size: 42 μm (< 80 μm (Van Rietbergen et al., 1995), X-ray tube potential (peak): 60 kVp, Integration time: 1680 ms per slice (Bouxsein et al., 2010) with a low dose of radiation (Genant et al., 1996). According to the literature (Follet et al., 2005; Syahrom 2011; Yeni et al., 2011, Garrison et al., 2011; Nazarian et al., 2011), 14 architectural parameters of cancellous bones were selected and computed using BoneJ (Doube et al., 2010) from the DICOM files. Parameters were divided into four groups: architectural descriptors of geometry, topology, connectivity and anisotropy. Parameters, units, and groups are presented in Table 1.

2.3. Experimental technique and mechanical properties

Experiments were performed as described in a previous study (Laporte et al., 2009). Twenty-four specimens underwent dynamic loading using a Split Hopkinson nylon Pressure Bar system (Liu et al., 2014) (SHPB, Fig. 1). A SHPB system operates under the following principles: a striker first impacts the free end of the input bar; this impact generates a compressive wave, known as the incident wave, which propagates through the input bar. When it reaches the input bar–specimen interface, part of the wave is reflected back along the input bar as a tensile wave, referred to as the reflected wave. The remaining part of the wave is transmitted, through the specimen to the output bar and is known as the transmitted wave. The relative magnitudes of the waves, registered through gages, relate to the material properties of the specimen at high strain rate. The data analysis for the SHPB tests was conducted using the DAVID software (Gary, 2005).

Samples were divided for two tests series with different applied boundary conditions: typical compression tests without confinement for 12 specimens (D, *ca* 1000 s^{-1}) and confined dynamic tests for the 12 remaining specimens (CD, *ca* 1500 s^{-1}). For the latter, bone was placed in an aluminum confined cell (Fig. 2, inner diameter: 41 mm, *i.e.*, the bars diameter, to only test the bone). Dimensions were chosen to limit the radial expansion of the structure and the flow of bone marrow. Then a compromise has been made in order to keep the specimen free of internal stresses and directly in contact with the cell, in order to be close to *in-vivo* boundary condition with cortical bone.

The mechanical properties were identified by an automatic process: ultimate stress and strain were determined at the maximum value of stress history (see Fig. 3 left) while the apparent Young's modulus was calculated based on the gradient of the stress *versus.* strain graph for the middle one third of the strain range between zero and ultimate strain (see Fig. 3 right). Initial strain was set as the left-shifting of the stress–strain curve based on the linear extrapolation to zero stress (Boruah et al., 2013).

2.4. Data analysis

Kruskal–Wallis test was used to determine if there is a difference between the 2 groups of micro-architectural parameters. Mann–Whitney statistical test (non-parametric hypothesis test) was performed for mechanical parameters to appraise the influence of boundary conditions. After validating the previous studies, the Spearman statistical test (non-parametric one-way analysis of variance by ranks) was used to highlight correlations between architectural and mechanical parameters. A p-value of 0.05 was chosen as the upper threshold of significance. All statistical tests were performed using XLSTAT.

3. Results

3.1. Microstructure and mechanical properties

To perform the analysis study, the variability of experimental and architectural data had been investigated. An illustration is presented in Fig. 4. This representation allows exploring the dataset mean (illustrated with a cross), the median (straight line), the 1st quartile and the 3rd quartile (ends of the box), the highest and lowest values (ends of the whiskers) and outliers if any (individual points). Medians, interquartile ranges, extremes values, mean values and standard deviations of all microstructure parameters for the loaded specimens are given in Table 2. Values for each specimen are tabulated in Supplementary information. Architectural parameters were consistent between the two sets of testing groups (minimum p-value for Kruskal–Wallis test: 0.44).

Architectural values have been found relevant to the literature dedicated to bovine samples (Syahrom, 2011; Halgrin, 2009; Garrison et al., 2011).

The two different groups can be compared as the maximum p-value is 0.006 for the Mann–Whitney test.

Descriptive statistics of mechanical parameters for the 24 compressed specimens are provided in Table 2. Supplementary information provides the actual test data for all the loaded specimens. Data published for bovine cancellous bone loaded to high strain rates (Higgins, 2008; Halgrin, 2009) set the same magnitude of individual variation.

As no work had been reported regarding confined dynamic loading, no comparison can be made; however, the confinements lead to a temperature elevation and added to the marrow influence. It appears that both apparent Young's modulus and ultimate stress increase in regard to non-confined testing (Halgrin, 2009).

3.2. Links between mechanical and microstructural parameters

As mentioned above, it is well known that mechanical response is linked to architecture. A bone presenting a higher bone

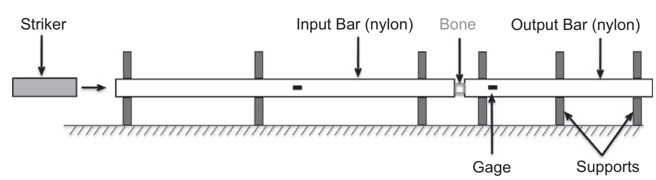

Fig. 1. Split Hopkinson Pressure Bar system used for high strain rate compressive testing.

Table 1
Summary of architectural parameters.

Family	Parameter	Description	Unit
Geometry and morphology	BS	Bone surface	mm^2
	BV	Bone volume	mm^3
	BV/TV	Bone volume/total volume	%
	Tb.Th	Mean thickness of trabeculae	mm
	Tb.Sp	Trabecular separation	mm
	Conn.D	Connectivity density or number of trabeculae per unit volume	$/mm^3$
Topology	FD	Fractal dimension	
	SMI	Structure model index	
Connectivity	Nd.Nd	Average branch length	$/mm^3$
	N.Nd	Number of junctions	$/mm^3$
	N.Jnv	Number of junction voxel, *i.e.* number of voxels labeled as being in a junction with more than 2 neighbors	$/mm^3$
	N.Slv	Number of slab voxel, *i.e.* number of voxels labeled as being in a junction with 2 neighbors	$/mm^3$
	N.Tp	Number of triple point, *i.e.* number of junctions with 3 branches	$/mm^3$
	N.Qp	Number of quadruple point, *i.e.* number of junctions 4 branches	$/mm^3$
Anisotropy	DA	Degree of anisotropy	
	MIL	Mean intercept length	mm