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The micro-structure of bone trabecular fracture: An inter-site study

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ABSTRACT

Trabecular bone fracture represents a major health problem, therefore the improvement of its assessment is mandatory for the reduction of the economic and social burden. The micro-structure of the trabecular bone was found to have an important effect on trabecular mechanical behavior. Nonetheless, the high variability of the trabecular micro-structure suggests a search for the local characteristics leading to the fracture. This work concerns the study of the local trabecular fracture zone and its morphometrical characterization, aiming to prediction of the probable fracture zone.

Ninety micro-CT datasets acquired before and after the mechanical compression of 45 trabecular specimens were analyzed. Specimens were extracted from the lower limbs of two donors: 4 femora and 4 tibiae. A previously validated tool for the identification of the 3D fracture zone was applied and the local fracture zone was identified and analyzed in all the specimens. Fifteen morphometrical parameters were extracted for each local fracture zone. Standard statistical non-parametric analysis was performed to compare fractured and un-fractured zones together with a classification analysis for the prediction of the fracture zone.

The statistical analysis showed strong statistical difference in the micro-structure of the trabecular fractured zone compared to the un-fractured one. Ten out of 15 measured parameters, like SMI, Tb.Th, BV/TV, off-axis angle, BS/BV and others, showed a statistical difference between full 3D fractured and un-fractured zones. Nonetheless, a satisfactory classification of the fractured zone was possible with none of the identified parameters. On the other hand, a total classification accuracy of 95.5% was presented by the application of a linear classifier based on a combination of the most representative parameters, like BS/BV and the off-axis angle.

The study points out the local essence and peculiar characteristics of the fracture zone, it highlights the weakness of some parameters in discriminate between fractured and un-fractured zones and encourage focussing the future studies over the local fracture zone itself with the aim to identify objective differences that could one day lead to the improvement of clinical assessment of fracture risk.

Introduction

Fractures of trabecular bone represent a major health problem to the aging population. “In 2000, the number of osteoporotic fractures was estimated at 3.79 million” [1] and a future trend of 3.5 million fractures per year is estimated based on data for 2010 [2]. Thus, correct prediction of trabecular bone fracture risk is necessary in order to prevent traumatic events and related medical efforts. Local micro-structure of trabecular bone seems to play an important role in the definition of mechanical properties [3–6]. A validation study recently suggested fracture zone itself to be a local event [7]. Nonetheless, the local fracture zone and its peculiar characteristics have not been deeply studied.

The standardized analysis for the clinical assessment of fracture risk is based on the evaluation of global bone mineral density (BMD), measured on a specific anatomical site by means of dual X-ray absorptiometry. Many studies have indicated BMD as the most important parameter for determining the tissue's mechanical properties. However, using only the BMD to identify individuals at higher risk of fracture is not always reliable [8,9]. The World Health Organization suggests the use of FRAX® as a tool for the fracture risk assessment [10–13]. The tool is based on the use of indirect parameters of bone quality (e.g. country of residence, age, family and personal history). In *in-vitro* studies performed at micro level, the trabecular bone density is usually measured as bone volume fraction (BV/TV), due to its linear relation with ash density [14], and it is the most used parameter for in-vitro determination of mechanical properties [15–18]. Nonetheless, the assessment of bone fracture risk using BV/TV, BMD or FRAX® resulted in errors ranging from 20% to 40% both in *in-vitro* and in clinical studies [4,11,19,20]. The fracture zone should be directly studied in order to extract specific parameters that clearly represent the bone quality of the critical zone, thus potentially reducing errors in the assessment of trabecular bone fracture.

Trabecular bone micro-structure was found to have an important effect on bone strength [15,21]. A clear analysis of the trabecular bone micro-structure is currently limited to *in-vitro* analyses using high

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resolution micro-tomographic devices. Nonetheless, high resolution analysis has the potential to direct the development of future clinical tools [22]. Using micro-level acquisitions it is possible to analyze the alterations in trabecular bone micro-structure that may affect its mechanical behavior [23,24]. Micro-tomographic (micro-CT) devices are well known and tested facilities for the analysis of bone trabecular tissue [25–30]. Over time, a great number of studies have used micro-CT analyses to identify changes in trabecular bone structure [27,31–33]. The most of the micro-structure analyses suggest BV/TV as best descriptor of the mechanical behavior, but fail in giving a uniform law defining the trabecular strength [18]. Many structural parameters were explored to describe the mechanical behavior of trabecular bone [16,34–36] but the involvement of each parameter in the failure of the tissue was not always evident. Finally, Lochmuller et al., in [37] asserts that "under experimental conditions of mechanically testing entire bones, bone microstructure does not improve prediction of mechanical failure", actually suggesting the use of micro-structure not useful in the clinical fracture risk assessment. Some of the mentioned contradictory results can be explained by unsuitable control of variability of trabecular micro-structure during the experiments. Studies have shown that trabecular micro-structure is highly variable and influence the mechanical behavior [38–40]. The assumption of uniformity in a structure that actually shows variability can therefore lead to mistakes, increasing the error of the measurements or giving misleading results because working under a hypothesis that is not verified. The description of mechanical behavior can only be accurate if the structure analyzed is homogeneous [41]. In *in-vitro* cases the assumption of uniformity was addressed performing local analysis of the trabecular micro-structure. Those studies showed accurate description of the mechanical behavior [3–5].

Nonetheless, being able to describe the mechanical behavior of trabecular specimens and being able to predict the fracture event are two different problems. In Perilli et al. [4], 50 specimens were extracted from only one anatomical site (femoral head). All the specimens were extracted with the trabecular structure always oriented along the principal load direction. In this controlled scenario a 5% error in the description of the mechanical behavior was reported using local BV/TV. Nonetheless, the error increased to 17% when the same parameter was used to identify the fracture zone [4], suggesting the need for deeper analysis of the fracture zone.

The present work is focused on the study of the micro-structure of the trabecular fracture zone and its characteristics. The aim of the work is to highlight structural differences between fractured and un-fractured bone as a first step in the study of the fracture zone that can lead in the future to the prediction of the fracture event. The identification of specific morphometrical parameters and their potential to discriminate fractured and un-fractured zones, is proposed by the use of a linear classifier.

Material & methods

This study is based on the analysis of data previously acquired during the European project Living Human Digital Library (LHDL; IST-2004-026932). Data access is allowed to all the users (http://www.physiomespace.com). The whole data collection procedure was developed during the LHDL project and fully described in the web-site of the Biomedical Research Community: BiomedTown (https://www.biomedtown.org/biomed_town/LHDL/Reception/collection/). The full procedure is summarized below.

Four femora and four tibiae were obtained from two cadavers without skeletal disorders by means of a donor program during the LHDL European project. The samples had been embalmed using the modified Dankmeyer's method [42,43]. Tibiae and femora were cut into slices perpendicular to the longitudinal axis of the bone. The epiphyses were cut into 26 mm slices. Fifty-six (56) cylindrical trabecular specimens, with a diameter of 10 mm, were then extracted [14]. Typical extraction

Fig. 1. Extraction sites of trabecular specimens for femurae and tibiae.

sites are shown in Fig. 1. All specimens were scanned by means of a micro-CT (model Skyscan 1072, Bruker microCT, Kontich, Belgium) with an isotropic voxel size of 19.5 μm, using a previously published protocol [44,45]. All specimens underwent compressive testing (model Mini bionix 858, MTS Systems Corp., Minneapolis, MN, USA). Each specimen was cemented directly onto the testing machine. The free length of each specimen was 20 mm [4,5,46]. The same acquisition procedure was performed both before (pre-failure dataset) and after the mechanical testing (post-failure dataset). In each set, the final analyzed Volume of Interest (VOI) was a cylinder of 991 micro-CT cross-sections (slices) equal to 19.3 mm height and 9 mm in diameter [4]. Hereafter, the analysis performed over this VOI will be called *global analysis* [5]. A fixed threshold was used for the segmentation of trabecular structure from marrow, as previously reported [45,46].

The whole study was developed in four steps aiming to analyze the fracture zone (Fig. 2):

Image processing block: Identification of the fracture zone
Calculation block: morphometric analysis of the structure
Comparison block: statistical analysis
Prediction block: classification analysis

Image processing block: Identification of the Fracture Zones

The trabecular fracture zone was defined as the region presenting brittle fracture or plastic deformation of at least one trabecula. A previously validated and published scheme for the automatic identification of the trabecular fracture zone was applied to all datasets [7]. The scheme is briefly described below (Fig. 3).

Automatic identification of the fracture zone was performed using a three-dimensional (3D) automatic rigid registration method. The registration was applied to both acquired datasets; the pre-failure dataset and the post-failure dataset. Two subsets of the post-failure dataset were defined by their position relative to the fracture zone; accordingly referred to as the upper and lower subsets. The proposed registration method was then applied twice: the first registration involves the upper subset of the post-failure dataset with the pre-failure set whereas