Brillouin micro-spectroscopy of subchondral, trabecular bone and articular cartilage of the human femoral head

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Abstract: Brillouin micro-spectroscopy is applied for investigating the mechanical properties of bone and cartilage tissues of a human femoral head. Distinctive mechanical properties of the cartilage surface, subchondral and trabecular bone are reported, with marked heterogeneities at both micrometric and millimetric length scales. A ubiquitous soft component is reported for the first time, characterized by a longitudinal modulus of about 4.3 GPa, possibly related to the amorphous phase of the bone. This phase is mixed, at micrometric scales, with a harder component, ascribed to mineralized collagen fibrils, characterized by a longitudinal modulus ranging between 16 and 25 GPa.

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1. Introduction

Bone is a lively tissue, whose structure is remodeled by function, age, gender differences, etc., depending on both mechanical stimuli and by mineral, metabolic and hormonal homeostasis. Understanding biomechanical properties of this tissue at different spatial scales is crucial, not only to figure out how bones and joints can resist to stresses but also to detect early changes in their functionality which can lead to pathologic conditions. Bone is a hierarchically-organized tissue [1], with a mineralized extracellular matrix (ECM) that contains about 25% organic matrix, 5% water, and 70% inorganic mineral compound. The organic matrix is composed of bundles of mineralized collagen fibers assembled together with non-collagenous proteins (NCPs), such as osteocalcin, osteopontin, bone-sialoprotein, glycosaminoglycans, and proteoglycans. At micrometric level of organization, mammalian adult bones are principally composed by lamellar bone [2,3]. Each lamella (3-7 microns), is made by two different materials: a predominant ordered phase, composed by arrays of mineralized collagen fibers organized in precisely oriented patterns, and a disordered phase, composed by a mineralized ECM with poorly oriented collagen fibers and a great amount of NCPs, proteoglycans, and water. The disordered phase is made by a lacuno-canalicular network, with mechano-sensing and mineralization functions [4] performed by bone cells (osteoblasts, osteoclasts, and osteocytes) which can survive and communicate one with the others within this disordered structure. Depending on the spatial position and the timing of bone formation and remodeling, several lamellae can adopt a number of complex structural motifs [5,6]. The inner structure of long bones, such as the femur, is composed by different sub-structures: a compact bone body, a small portion of spongy tissue (trabecular or cancellous bone) and bone marrow. The ends of long bones (Fig. 1(a)) are usually covered by cartilage, a highly specialized connective tissue. It is composed by specialized cells (chondrocytes), which produce an abundant extracellular matrix, very rich in proteoglycans,
water and fibers, such as collagen and elastin [7], providing a smooth, lubricated surface, which is crucial to prevent the friction between opposite bones and facilitate the transmission of loads. Cartilage and bone tissues are joint to form an elegant and sophisticated functional osteochondral unity. Pathological changes in the mechanical properties of one of these components, at one of the above described spatial scales, can compromise the integrity and functionality of the whole anatomic unit. Currently, mechanical properties of joints at the level of articular cartilage and subchondral bone can be studied by means of quantitative ultrasounds (QUS) [8] which can give global information on bone elasticity but does not allow to study the microscale level because of the relatively large irradiation area. Moreover, nanoindentation and scanning acoustic microscopy (SAM) techniques have been employed to measure bone hardness and acoustic impedance at tens/hundreds of micrometer scales [9,10]. However, there is a lack of techniques able to access the micrometer length scale, which is strategic to study lamellar and intra-lamellar properties. Moreover, these techniques have been used only in vitro and progress towards in-vivo investigations would be highly desirable. Promising results have recently obtained by means of micro-Brillouin on trabecular and cortical bovine bones [11,12], showing elastic anisotropies due to fibers orientation, also in comparison with SAM results. Different mammalian bones [13] and their induced regenerative properties have been recently characterized by micro-Brillouin. In this work, we test the potential of Brillouin micro-spectroscopy in a proof-of-concept study of the mechanical properties of the human femoral head.

2. Materials and methods

2.1 Sample description and preparation

![Diagram](image)

Fig. 1. (a) 3D rendering image of a human femoral head with the site of inferomedial region from which the sample was selected. (b) Longitudinal section of the sample with subchondral bone (SB) and trabecular bone (TB). (c) Top view of the same section, showing the articular non-calcified cartilage surface (NCC). d) Typical Brillouin spectra collected from cartilage surface (blue), subchondral bone (red), and trabecular bone (black).

The sample analyzed in this work was obtained from a patient (male, 73 years of age) who underwent total hip arthroplasty. Damaged right femoral head was removed as a result of focal severe osteoarthritis (OA) in the superolateral region of the femoral head, and replaced with a prosthetic stem. After signing the informed consent, just after removal, a specimen of 1 cm in thickness and 1 cm in width was collected from the inferomedial region of the femoral head [14]. This was a healthy site without radiographic and macroscopic evidence of tissue degeneration, i.e. grade zero of Kellgren-Lawrence grading scale [15] and translucent cartilage with a bluish-white tinge typical of healthy chondral tissue. In this way, articular cartilage, subchondral bone and trabecular bone of healthy tissue were accessible to micro-Brillouin analysis. After the surgical procedure, the collected sample was processed as
described in Ref [16]. Briefly, the sample was fixed in a solution of 4% paraformaldehyde (PFA; Sigma-Aldrich) for 24 hours, washed in running tap water and distilled water and stored at room temperature in a solution of 70% ethanol, which does not destroy the sample due to prior PFA fixation. Before measurement, the sample was extracted from ethanol and dried in air.

2.2 Measurements and data elaboration

The micro-Brillouin spectroscopic set-up here employed, characterized by 2μm spot size, and the sample installation are described in Refs [17,18]. 172 spectra were collected at random in the articular non-calcified cartilage surface (Fig. 1(c)), 196 spectra in the subchondral bone, down to 3 mm from the edge of the section, and 218 spectra in the trabecular bone (Fig. 1(b)). Each spectrum was recorded for about 150 s. Typical spectra are reported in Fig. 1(d). Each spectrum shows two main peaks, one at low frequency (green box) and the other at high frequency (magenta box). Relevant for the elastic characterization of the sample is the frequency shift \( \nu \) of these peaks. In fact, in back-scattering experiments, the longitudinal elastic modulus \( M \) of the tissue can be obtained from \( \nu \) through the relationship

\[
2\pi^2 \nu^2 = \frac{n^2}{\rho \lambda}
\]

where \( \lambda \) is the wavelength of the laser, \( \rho \) is the mass density and \( n \) the refractive index of the sample. In the following we assume \( \rho = 2 \text{ gr/cm}^3 \) and \( n = 1.55 \) [19] and a constant ratio \( \rho / n^2 \) through the whole sample, which has been found as a reasonable approximation in different tissues [20]. Finally, to get the values of \( \nu \), the choice of any particular spectral function for fitting the line-shape of Brillouin peaks in Fig. 1(d) would be quite unjustified due to the mixing of homogeneous and heterogeneous mechanisms of broadening. For this reason, we adopt a model-independent estimate of the average frequency shift of each peak through calculation of the first spectral moment [21], i.e.

\[
\bar{\nu} = \frac{\sum_i I_i \nu_i}{\sum_i I_i}
\]

where the index \( i \) spans spectral channels in the range 4-13 GHz and 13-32 GHz for the low-frequency (\( \nu_L \)) and high-frequency (\( \nu_H \)) modes, respectively.

3. Results and discussion

On the basis of what is already known about the morphology of the bone, the low-frequency peak in Fig. 1(d) can be tentatively attributed to the soft component of the tissue, related to a disordered phase of thin-poorly oriented collagen fibers, proteoglycans, and water. On the other hand, the high-frequency peak, with a peculiar frequency distribution, can be related to wide bundles of mineralized fibers characterized by different degrees of mineralization, ranging from the absence of mineralization in collagen fibers on the articular surface up to the high mineralized structures presents in the bone tissue. From 586 random measurements performed within different areas in the sample, we obtained the distribution of values for the frequency shift of the low and high-frequency peaks that are reported in Fig. 2, left panel.

3.1 Articular cartilage

The articular cartilage surface is characterized by a distribution of low-frequency Brillouin peaks (Fig. 2(a)) falling in the range 8 – 8.9 GHz, centered at about 8.56 GHz, corresponding to a longitudinal elastic modulus of about 4.3 GPa. The high-frequency mode shows a very narrow dispersion (Figs 2(d)), with values between 17.5 and 18.5 GHz and an average value of about 17.7 GHz, corresponding to an elastic modulus of about 18.5 GPa. This high-frequency mode can be tentatively attributed to tight collagen fibers, which are arranged between the chondrocytes and the amorphous phase of peptidoglycans and water. This region of the sample is the most elastically homogenous (narrow distributions in Figs. 2(a),(d)), in accordance with the characteristics of articular cartilage, which is expected to show a gradient in fibers/amorphous phase ratio and orientation in depth and not in the plane.
Fig. 2. Left panel: distribution of the first spectral moments of Brillouin peaks in the articular cartilage surface (blue) subchondral bone (red), and trabecular bone (black). Frequency shifts of low frequency modes are reported in panels a), b) and c) and those of high frequency modes in panels d), e) and f). Right panel: frequency shifts of low frequency ($\nu_L$) and high frequency ($\nu_H$) modes collected from trabecular bone, showing an almost linear correlation, with $r = 0.79$.

3.2 Subchondral bone

Subchondral bone spectra are characterized by a low-frequency mode, which covers values from 7.9 to 8.9 GHz (Fig. 2(b)), very similar to that of the articular cartilage. On the other hand, the high-frequency mode shows values comprised from 21 up to 25 GHz and centered at about 23 GHz (Fig. 2(e)), corresponding to an elastic modulus of about 31.2 GPa. This is the largest value for the longitudinal modulus found in our sample, suggesting that this part of the bone is characterized by the highest rate of ordered mineralized fibers, giving to the tissue its characteristic stiffness and mechanical resistance.

3.3 Trabecular bone

Trabecular bone is the most heterogeneous region of the sample. The low-frequency mode, which covers a range from 7.8 GHz to 8.4 GHz (Fig. 2(c)), shows an overall softer behavior with respect to the other two regions. Moreover, the high-frequency mode (Fig. 2(f)) shows a bi-modal distribution in frequency with a first maximum at about 17.5 GHz ($M = 18$ GPa), close to that of the articular surface, and the second maximum at about 20 GHz ($M = 23.6$ GPa), which is intermediate between the articular surface and the subchondral bone. This mechanical heterogeneity is possibly due to the co-existence of regions characterized by different degrees of mineralization. In particular, softer regions are probably characterized by ordered collagen fibers not fully mineralized, while stiffer regions are made by ordered bundles of mineralized collagen fibers, but with a lower content on hydroxy-apatite crystals with respect to the subchondral bone. Moreover, it is interesting to notice that the frequency shifts of low and high-frequency modes show some degree of correlation: the stiffness of the mineralized tissue tends to increase together with that of the softest component (Fig. 2).

3.4 Comparison with elastic moduli measured by other techniques

Thought Brillouin scattering gives the longitudinal modulus rather than the Young modulus, it is interesting to notice that our results are consistent with those already obtained by both nano-indentation and scanning acoustic microscopy (SAM), namely an elastic modulus of trabecular lamellae in the TB zone considerably lower than that of the subchondral bone (SB). In particular, Turner et al. [10] reported that Young’s elastic modulus on longitudinal and transverse directions of the human femoral diaphysis are 20.55 and 14.95 GPa, respectively,
when measured by SAM and about 23.45 and 16.58 GPa when measured by nanoindentation. Also, Young’s elastic modulus obtained on the trabecular bone of the human distal femur showed a similar trend, with a value of about 17.50 GPa measured by SAM and 18.14 GPa measured by nanoindentation. On the other hand, Ashman et al. [22] reported lower values of about 13.0 GPa obtained on the same type of samples by ultrasonic techniques. Other nanoindentation studies performed on the wet sections of human femur, conducted by Zysset et al. [23] in the femoral neck reported values of 15.8 GPa, 17.5 GPa and 11.4 GPa for osteonal, interfibrillar and trabecular lamellae, respectively, and values of 19.1 GPa and 21.2 GPa for osteonal and interfibrillar lamellae respectively in the femoral mid-diaphysis. Finally, Chevalier et al. [24] reported values for Young’s elastic modulus from 19.6 to 21.8 GPa on the dried trabecular part of the three different femoral heads.

With respect to previous mechanical studies of femoral bones, we stress that our micro-Brillouin investigation has provided a piece of new important information: both the subchondral bone plate and the trabecular bone core present an additional soft component, characterized by a longitudinal modulus of about 4.3 GPa, highlighting the bio-composite mechanical nature of the analyzed bone tissue. This is an anomalous feature with respect to previous mechanical investigations, which can be tentatively attributed to the presence of sub-micrometric soft heterogeneities in the sample (see Fig. 8, inset VII of Ref [4].). In fact, these heterogeneities can elude both SAM and nanoindentation measurements, averaged out by the long wavelength of ultrasounds and the 1-5 μm resolution of indenters [10], but can be revealed by the ~200 nm wavelength of Brillouin micro-spectroscopy.

4. Conclusions

Brillouin microspectroscopy has been applied for the first time to the study of the mechanical properties of the human femoral head. Measurements performed at random on cartilage surface, subchondral and trabecular bone have given evidence of marked mechanical heterogeneity. In all the investigated regions, bimodal spectra have been revealed, a clear signature of the coexistence of soft (4.3 GPa) and hard (16 and 25 GPa) regions within the few micrometers of the scattering volume. The soft component, never evidenced before, can be attributed to the propagation of acoustic phonons through the amorphous fraction of the bone. The hard region can be identified with mineralized collagen fibrils. The quite large distribution of elastic moduli in mineralized regions is usually attributed to mechanical anisotropies induced by different orientations of collagen fibrils. Measurements in progress in our labs on samples obtained by different donors, with and without fixation, confirm the generality of the coexistence of soft and hard regions. Their characteristic frequencies and relative intensities have the potential of giving precious mechanical information at the micrometric scale on the pathological condition of bones.

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

References

1. N. Reznikov, R. Shahar, and S. Weiner, “Bone hierarchical structure in three dimensions,” Acta Biomater. 10(9), 3815–3826 (2014).
2. J.-Y. Rho, L. Kuhn-Spearing, and P. Zioupos, “Mechanical properties and the hierarchical structure of bone,” Med. Eng. Phys. 20(2), 92–102 (1998).
3. H. P. Schwarcz, D. Abueidda, and I. Jasiuk, “The Ultrastructure of Bone and Its Relevance to Mechanical Properties,” Front. Phys. 5, 39 (2017).
4. N. Reznikov, M. Bilton, L. Lari, M. M. Stevens, and R. Kröger, “Fractal-like hierarchical organization of bone begins at the nanoscale,” Science 360, eaao2189 (2018).
5. N. Reznikov, R. Shahar, and S. Weiner, “Three-dimensional structure of human lamellar bone: the presence of two different materials and new insights into the hierarchical organization,” Bone 59, 93–104 (2014).
6. O. A. Tertuliano and J. R. Greer, “The nanocomposite nature of bone drives its strength and damage resistance,” Nat. Mater. 15(11), 1195–1202 (2016).
7. A. J. Sophia Fox, A. Bedi, and S. A. Rodeo, “The Basic Science of Articular Cartilage: Structure, Composition, and Function,” Sports Health 1(6), 461–468 (2009).
8. Y. Yamato, M. Matsuura, T. Otani, K. Yamazaki, and A. Nagano, “Distribution of longitudinal wave properties in bovine cortical bone in vitro,” Ultrasonics 44(Suppl 1), e233–e237 (2006).
9. J.-Y. Rho, M. E. Roy 2nd, T. Y. Tsui, and G. M. Pharr, “Elastic properties of microstructural components of human bone tissue as measured by nanoindentation,” J. Biomed. Mater. Res. 45(1), 48–54 (1999).
10. C. H. Turner, J. Rho, Y. Takano, T. Y. Tsui, and G. M. Pharr, “The elastic properties of trabecular and cortical bone tissues are similar: results from two microscopic measurement techniques,” J. Biomech. 32(4), 437–441 (1999).
11. M. Matsuura, R. Tsubota, M. Kawabe, and K. Fukui, “Application of a micro-Brillouin scattering technique to characterize bone in the GHZ range,” Ultrasonics 54(5), 1155–1161 (2014).
12. K. Fukui, S. Takayanagi, D. Suga, and M. Matsuura, “Measurement of Wave Velocity in Cortical Bone by Micro-Brillouin Scattering Technique: Effect of Bone Tissue Properties,” Jpn. J. Appl. Phys. 51(75), 07GF20 (2012).
13. D. Aklilbekova, V. Ogay, T. Yakupov, M. Sarsenova, B. Umabayev, A. Nurakhmetov, K. Tazhin, V. V. Yakovlev, and Z. N. Utegulov, “Brillouin spectroscopy and radiography for assessment of viscoelastic and regenerative properties of mammalian bones,” J. Biomed. Opt. 23(9), 1–11 (2018).
14. D. Dallari, G. Pignatti, C. Stagni, G. Giavaresi, N. Del Piccolo, N. Rani, F. Veronesi, and M. Fini, “Total Hip Arthroplasty With Shortening Osteotomy in Congenital Major Hip Dislocation Sequelae,” Orthopedics 34(8), e328–e333 (2011).
15. J. H. Kellgren and J. S. Lawrence, “Radiological Assessment of Osteo-Arthrosis,” Ann. Rheum. Dis. 16(4), 494–502 (1957).
16. F. Veronesi, G. Giavaresi, M. Fini, G. Longo, C. A. Ioannidu, A. Scotto d’Abusco, F. Superti, G. Panzini, C. Misiono, A. Palattela, P. Selleri, N. Di Girolamo, V. Garbarino, L. Politi, and R. Scandurra, “Ossointegration is improved by coating titanium implants with a nanostructured thin film with titanium carbide and titanium oxides clustered around graphitic carbon,” Mater. Sci. Eng. C 70(1 Pt 1), 264–271 (2017).
17. F. Scarponi, S. Mattana, S. Corezzi, S. Caponi, L. Lopez, P. Sassi, A. Morresi, M. Paolantoni, L. Urbanelli, C. Emiliani, L. Roscini, L. Corte, G. Cardini, F. Palombo, J. R. Sandercock, and D. Fioretto, “High-Performance Versatile Setup for Simultaneous Brillouin-Raman Microspectroscopy,” Phys. Rev. X 7, 031015 (2017).
18. S. Mattana, M. Mattarelli, L. Urbanelli, K. Sagini, C. Emiliani, M. D. Serra, D. Fioretto, and S. Caponi, “Non-contact mechanical and chemical analysis of single living cells by microspectroscopic techniques,” Light Sci. Appl. 7(2), 17139 (2018).
19. I. M. Berke, J. P. Miola, M. A. David, M. K. Smith, and C. Price, “Seeing through Musculoskeletal Tissues: Improving In Situ Imaging of Bone and the Lacunar Canalicular System through Optical Clearing,” PLoS One 11(3), e0150268 (2016).
20. M. Kawabe, K. Fukui, M. Matsuura, M. Gronke, A. Saied, Q. Grimal, and P. Laugier, “Comparative investigation of elastic properties in a trabecula using micro-Brillouin scattering and scanning acoustic microscopy,” J. Acoust. Soc. Am. 132(1), EL54–EL60 (2012).
21. D. Fioretto, S. Caponi, and F. Palombo, “Brillouin-Raman mapping of natural fibers with spectral moment analysis,” Biomed. Opt. Express 10(3), 1469–1476 (2019).
22. R. B. Ashman, J. Y. Rho, “Elastic modulus of trabecular bone material,” J. Biomech. 21(3), 177–181 (1988).
23. P. K. Zysset, X. E. Guo, C. E. Hoffer, K. E. Moore, and S. A. Goldstein, “Elastic modulus and hardness of cortical and trabecular bone lamellae measured by nanoindentation in the human femur,” J. Biomech. 32(10), 1005–1012 (1999).
24. Y. Chevalier, D. Pahr, H. Allmer, M. Charlebois, and P. Zysset, “Validation of a voxel-based FE method for prediction of the uniaxial apparent modulus of human trabecular bone using macroscopic mechanical tests and nanoindentation,” J. Biomech. 40(15), 3333–3340 (2007).