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Mechanical and mineral properties of osteogenesis imperfecta human bones at the tissue level

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ABSTRACT

Osteogenesis imperfecta (OI) is a genetic disorder characterized by an increase in bone fragility on the macroscopic scale, but few data are available to describe the mechanisms involved on the tissue scale and the possible correlations between these scales. To better understand the effects of OI on the properties of human bone, we studied the mechanical and chemical properties of eight bone samples from children suffering from OI and compared them to the properties of three controls. High-resolution computed tomography, nanoindentation and Raman microspectroscopy were used to assess those properties. A higher tissue mineral density was found for OI bone (1.131 gHA/cm^3 vs. 1.032 gHA/cm^3, $p = 0.032$), along with a lower Young's modulus (17.6 GPa vs. 20.5 GPa, $p = 0.024$). Obviously, the mutation-induced collagen defects alter the collagen matrix, thereby affecting the mineralization. Raman spectroscopy showed that the mineral-to-matrix ratio was higher in the OI samples, while the crystallinity was lower, suggesting that the mineral crystals were smaller but more abundant in the case of OI. This change in crystal size, distribution and composition contributes to the observed decrease in mechanical strength.

Introduction

Osteogenesis imperfecta is a collagen disease caused, in most cases, by a genetic disorder that primarily affects the genes COL1A1 and COL1A2, which encode collagen type I chains. OI pathology cases are split into four types according to the Sillence classification [1]. The OI types are associated with different levels of disease severity. In this classification system, type I corresponds to the mildest form, type II corresponds to the perinatal lethal form, type III corresponds to the most severe form compatible with life, and type IV is an intermediate group between type I and type III. OI includes progressive skeletal deformities [2], fractures at all life stages, short stature [3–5], and often respiratory problems, dentinogenesis imperfecta [3] and musculoskeletal manifestations [6]. More details about the forms of osteogenesis imperfecta can be found in previous publications [3,7–9].

Studies on mutations involved in this disease [4,10–13] are very helpful from the perspective of developing a curative treatment, but because little is known about the disease mechanisms, current treatments aim principally at helping people live with the pathology. Moreover, only a few treatments exist, primarily orthopedic surgery and bisphosphonates. Patients can undergo surgery when they suffer from deformities or fractures [14], but only the bisphosphonates act to strengthen the bones by reducing the resorbing activity [15]. Those medicines, given orally [16,17] or intravenously [18,19], are commonly prescribed for children suffering from OI. This treatment allows a significant decrease in the fracture rate and improves ambulation or mobility [16,17].

Although the effects of OI on bone are substantial, predicting the fracture risk remains challenging. This pathology, also known as "brittle bone disease", has considerable effects at the whole-bone scale, including short stature and bone deformities. Several studies have tried to apply assessment tools such as dual energy X-ray absorptiometry (DXA) to fracture forecasting in OI [19–22]. However, they showed that the bone modifications made it difficult to interpret the results [20,21]. These modifications have also been observed at the microstructure scale; for instance, the cortices were thinner and the trabecular bone volume per tissue volume was decreased in patients with OI [23–25].

The tissue characteristics are also altered at the ultrastructural scale. Histomorphometric analyses of the collagen fibril diameters indicated that type I collagen fibrils in OI bone were different in diameter compared with those in normal bone and that the structure of collagen fibrils in the osteoid was not uniform [26–28]. Such abnormal fibrillogenesis observed in type III would also disturb the sites of scaffolding and nucleation for normal mineralization [29]. Mineral density, shape, size and mineral composition of crystals in OI bone are then modified due to collagen abnormalities. These differences have already

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been observed at different scales with different techniques [23,26,28,30–34]. However, the consequences of these mineralization abnormalities on the local mechanical properties in OI bones remain controversial.

Nanoindentation is now the reference technique for determining biomechanical properties at the micrometric scale [35,36]. In this technique, a diamond-tip indenter is displaced into the surface of a specimen, allowing local measurements of Young's modulus and hardness of the material on a small, micrometric area. Determination of Young's modulus and hardness of human OI bones was investigated using nanoindentation by several authors. Fan et al. [37] reported no significant difference in elastic modulus between moderate (type IV) and severe OI (type III). However, Albert et al. recently showed that individuals with osteogenesis imperfecta type I had higher modulus and hardness than did those with type III [38].

Previous studies performed at the tissue scale have individually shown that OI leads to a modification of bone mineral density, elastic modulus, and crystal size, but these properties have never been studied together in human patients. The aim of this study was, therefore, to investigate the tissue qualities of bones from patients suffering from osteogenesis imperfecta and to compare them to the properties of control bones. Bone mineral density, local mechanical properties and bone composition were assessed with high-resolution X-ray tomography, nanoindentation and Raman microspectroscopy, respectively.

Materials and methods

The present study investigated the mechanical and chemical properties of bone tissue from patients with OI in comparison to controls. This experimental study included high-resolution X-ray computed tomography, nanoindentation and Raman microspectroscopy measurements.

Specimens

Bone specimens were collected over one year from young individuals with and without OI pathology. OI donors were seven pediatric patients (four males, three females) between 4 and 16 years old with OI from mild to severe forms, who underwent routine surgery at Necker Hospitals for Children — Paris for fracture repair or deformity correction of lower extremity long bones. They sustained a minimum of six fractures and were all treated with bisphosphonates. The control group was composed of three donors (one male, two females) without bone pathology who underwent routine surgery at Necker Hospitals for Children — Paris for fracture repair. Donor ages and BMI did not differ significantly between the two groups ($p = 0.23$ and $p = 1$, respectively). Eight cortical bone specimens from OI donors and three cortical bone specimens from healthy donors were collected from long bones. Two samples were from the same patient, but they were from both femurs in a patient who underwent surgery twice, two months apart. The study was conducted according to the Institutional Review Board recommendations (IRB 00003835 2010/28NI). The samples were stored at −20 °C until testing.

Sample preparation

All bone samples were cut with a diamond saw (Secotom-15, Struers A/S, Ballerup, Denmark) into small prisms of various sizes that depended on the amount of bone. The analyzed surfaces used for nanoindentation and Raman measurements were ground with sandpaper (grit #2400) and then polished using 1-μm diamond powder and a water-based lubricant. The normal of this surface was parallel to the osteonal axis.

High-resolution X-ray computed tomography

Each bone sample was immersed in a physiologic saline solution (NaCl 0.9%) within a cryotube and imaged by high resolution X-ray computed tomography (Phoenix Nanotom S, GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) equipped with a high-power nanofocus tube with molybdenum target. Projection images on a CCD camera were obtained at 70 kV and 130 μA with a resolution of 4 μm. A rotation of 0.18° was used between each image acquisition, providing a series composed of 2000 projection images. Then, from this series of projection images, the software Phoenix Datosx 2 (GE Sensing & Inspection Technologies GmbH, Wunstorf, Germany) was used to reconstruct a stack of 2-D sections for each bone sample and stored as TIFF files, with indexed gray levels ranging from 0 (black) to 255 (white). Two phantoms with 0.25 g/cm^3 and 0.75 g/cm^3 of hydroxyapatite for bone density calibration were imaged at the same time as the bone samples. Based on the maximum of the gray level distribution of the phantoms, a linear relationship between gray level and mineral density was deduced. The tissue mineral density was then calibrated using this linear relationship.

Nanoindentation

Nanoindentation tests were performed on sample surrounded by a physiological saline solution at the ambient temperature using a commercial nanoindenter (Agilent Nanoindenter G200, ScienTec, Les Ulis, France). Fused silica was used for calibration of the Berkovich diamond tip contact surface. Indentation tests were performed on a grid covering the maximum surface of the sample, producing a minimum spacing of 150 μm transversally from the osteon direction. A constant strain rate of 0.05 s^{-1} and a maximum depth of 2000 nm were imposed. The Continuous Stiffness Measurement (CSM) method allows a determination of Young's modulus and the hardness as functions of the displacement into the surface. In the present study, Oliver and Pharr's method was used [39] with the assumptions for a linear elastic isotropic material. The elastic properties of the diamond indenter were $\nu_i = 0.07$ and $E_i = 1131$ GPa. Moreover, the bone was assumed isotropic with a 0.3 Poisson ratio [40,41]. Young's modulus and the hardness for each point were measured on the plateau between 600 and 1200 nm. Indentations localized in holes or resulting in poor curves were not included in the analysis. The number of points per sample was at least 50 for the controls and 25 for the OI samples.

Raman spectroscopy

Raman spectroscopy (LabRAM HR 800, Horiba Jobin Yvon, Villeneuve d'Ascq, France) was used in wet conditions on the same sample surface used for nanoindentation. This technique uses Raman (inelastic) scattering to obtain information on the composition of the material; for more information on the principle, see the works of Morris and Mandair [42]. A 785-nm laser was used to excite the electrons within the material. The 50× objective and the numerical aperture of 0.75 produced a laser spot of approximately 2 μm in diameter. The acquisitions were made on the spectral range of 750 cm^{-1}–1750 cm^{-1}, with an integration time of 45 s and one accumulation. Five spectra were acquired for each point, and 30 points were measured for each of the eleven samples. LabSpec software (Horiba Jobin Yvon, Villeneuve d'Ascq, France) was used to despike the spectra and subtract the background. Then, the five spectra per point were averaged, and the resulting spectrum was smoothed using a sliding average over three points algorithm in a Matlab (The MathWorks Inc., Natick, Massachusetts, USA) routine.

Three maximum intensity ratios were calculated from the smoothed spectrum (Fig. 1), i) the mineral-to-matrix ratio, as the ratio between the ν_1PO_4 (961 cm^{-1}) and Amide I (1667 cm^{-1}) maximum intensities, describing the mineral content compared to the collagen matrix; ii) the carbonate-to-phosphate ratio, as the ratio between the CO_3 (1075 cm^{-1}) and the ν_1PO_4 (961 cm^{-1}), conveying the substitution rate; and iii) the ratio between 1667 cm^{-1} and 1685 cm^{-1}, described by different authors [42–44] as the ratio between the non-reducible trivalent (mature) and the reducible divalent