Bone 69 (2014) 61–67

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Rapid Communication

Increased intra-cortical porosity reduces bone stiffness and strength in pediatric patients with osteogenesis imperfecta

V. Vardakastani [a], D. Saletti [a], W. Skalli [a], P. Marry [b], J.M. Allain [c], C. Adam [a,d,*]

[a] Institut de Biomécanique Humaine Georges Charpak, Arts et Metiers ParisTech (ENSAM), 151 Boulevard de l'Hôpital, 75013 Paris, France
[b] Service de Chirurgie Orthopédique et Réparatrice de l'enfant, Hôpital Armand Trousseau, 26, avenue du Docteur Arnold Netter, 75571 Paris Cedex 12, France
[c] Laboratoire de Mécanique des Solides, CNRS UMR7649, Ecole Polytechnique, 91128 Palaiseau Cedex, France
[d] School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology, GPO Box 2434, 2 George St, Brisbane, Australia

ARTICLE INFO

Article history:
Received 30 May 2014
Revised 28 August 2014
Accepted 2 September 2014
Available online 16 September 2014

Edited by: Sharmila Majumdar

Keywords:
Osteogenesis imperfecta
Bone mechanics
Bone microstructure
Intra-cortical porosity
Collagen alignment

ABSTRACT

Osteogenesis imperfecta (OI) is a heritable disease occurring in one out of every 20,000 births. Although it is known that Type I collagen mutation in OI leads to increased bone fragility, the mechanism of this increased susceptibility to fracture is not clear. The aim of this study was to assess the microstructure of cortical bone fragments from patients with osteogenesis imperfecta (OI) using polarized light microscopy, and to correlate microstructural observations with the results of previously performed mechanical compression tests on bone from the same source. Specimens of cortical bone were harvested from the lower limbs of three (3) OI patients at the time of surgery, and were divided into two groups. Group 1 had been subjected to previous micro-mechanical compression testing, while Group 2 had not been subjected to any prior testing. Polarized light microscopy revealed disorganized bone collagen architecture as has been previously observed, as well as a large increase in the areal porosity of the bone compared to typical values for healthy cortical bone, with large (several hundred micron sized), asymmetrical pores. Importantly, the areal porosity of the OI bone samples in Group 1 appears to correlate strongly with their previously measured apparent Young's modulus and compressive strength. Taken together with prior nanoindentation studies on OI bone tissue, the results of this study suggest that increased intra-cortical porosity is responsible for the reduction in macroscopic mechanical properties of OI cortical bone, and therefore that *in vivo* imaging modalities with resolutions of ~100 μm or less could potentially be used to non-invasively assess bone strength in OI patients. Although the number of subjects in this study is small, these results highlight the importance of further studies in OI bone by groups with access to human OI tissue in order to clarify the relationship between increased porosity and reduced macroscopic mechanical integrity.

Introduction

Osteogenesis imperfecta (OI) is a heritable disease that appears in one in 20,000 births. The disease is caused by a mutation of Type I collagen, and seven subtypes of OI have been identified [1]. The symptoms and severity of the disease vary between patients, but the main shared characteristic of OI is bone fragility, leading to a high risk of fracture. As human specimens of OI bone are difficult to obtain, limited information is available in existing literature regarding the mechanism of this decrease in bone mechanical integrity. A number of previous studies have performed nanoindentation testing on human OI bone specimens [2–6] and each of these studies has concluded that both the elastic modulus and hardness of the bone tissue itself are only marginally different to typical values for healthy cortical bone, despite the fact that it is known that OI significantly degrades the macroscopic mechanical behavior of the bone [5].

The fact that OI bone exhibits essentially normal stiffness and strength at the nanoscale suggests that the cause of the degraded macroscopic mechanical properties occurs at scales above that of the collagen/apatite nanostructure. That is to say, micro-morphological factors may contribute to the abnormal macroscopic behavior of OI cortical bone. For instance, there are suggestions in previous studies that abnormal collagen orientation and lamellar architecture affect bone mechanical integrity in OI, and decreased lamellar thickness and osteonal size have been reported in OI [7–10]. Moreover, the formation of micro-cracks and accumulation of micro-damage can also degrade mechanical behavior. Previous mouse studies [8,11] indicate that OI bone shows a greater propensity to accumulate micro-damage and to form linear micro-cracks than normal bone. Bulk degradation of bone properties due to micro-cracks would not necessarily be detected by nanoindentation, whereas in a macroscopic mechanical test they would behave as material defects. Thirdly, there is the observation that cortical

* Corresponding author at: Institut de Biomécanique Humaine Georges Charpak, Arts et Metiers ParisTech (ENSAM), 151 Boulevard de l'Hôpital, 75013 Paris, France.
E-mail address: clayton.adam@ensam.eu (C. Adam).

http://dx.doi.org/10.1016/j.bone.2014.09.003
8756-3282/

bone in OI becomes 'trabecularized' [12], so that although the matrix itself may not be mechanically compromised, the increased porosity could degrade macroscopic mechanical properties.

Given the apparent importance of microstructural alterations in OI, the aim of this study was to examine the microstructure of OI cortical bone in a series of biopsy specimens from human subjects using polarized light microscopy. Collagen fiber organization was qualitatively assessed, and areal intra-cortical porosity was calculated. Porosity was then compared to micro-mechanical stiffness and strength measured during previously performed compression tests on a subset of the specimens.

Materials and methods

Specimen acquisition

The human OI cortical bone specimens used in this study were harvested during surgical rodding procedures for the fixation of femoral and tibial fractures undertaken at Hospital Armand Trousseau in Paris, France. The study protocol was approved by the Hospital ethical committee and written parental consent was obtained for each patient. After harvest, the biopsy specimens were wrapped in saline soaked gauze and frozen at −18 °C prior to transport to the laboratory.

Group assignment

A subset of the specimens described in the present study had been subjected to mechanical compression testing after harvest as part of a prior (unpublished) study. These specimens were assigned to Group 1. Group 2 comprised the remaining specimens which had not been previously compression tested, thereby allowing assessment of microstructure in untested specimens (since mechanical compression to failure could potentially affect microstructure, even though the final compressive strain was generally only a few percent). Since the protocol for the mechanical compression tests mentioned above has not been previously published, it is described below. The reader is referred to [13] for further detail of the compression tests from the unpublished thesis. After mechanical compression testing of the specimens in Group 1, all specimens from both groups were prepared for polarized light microscopy as described in the section 'Resin embedding' below.

Mechanical compression testing of Group 1 specimens

Unconfined compression tests were performed on the specimens assigned to Group 1 in the present study using a uniaxial RAITH® testing device [14]. Since the surgically harvested biopsy fragments were of irregular size and shape, prior to testing each specimen was thawed and cut under constant irrigation into a parallelepiped shape using a diamond saw (Isomet Low Speed Saw, Buehler, USA). Due to the variation in size of the biopsy fragments, the resulting parallelepipeds also varied in size. Dimensions of the prepared specimens varied from 1.3 to 4.3 mm in width and thickness, and from 5.0 to 6.9 mm in length. When biopsy fragments were too thin to be securely held for cutting with the diamond saw, specimen preparation was achieved using manual polishing with successively finer polishing disks until a flat surface had been achieved. For each specimen, the cutting direction was chosen such that the faces of the parallelepiped test specimen were approximately parallel and perpendicular to the fabric direction of the host bone tissue respectively, as ascertained by visual inspection of the microstructure of the harvested fragments prior to cutting. Each specimen was then oriented in the uniaxial testing apparatus such that the applied loading was approximately aligned with the fiber direction (primary loading axis) of the bone *in vivo*. Prior to each compression test, black ink dots were applied to the side of the specimen to aid in subsequent image analysis using digital image correlation for strain determination, then the sample was preloaded with three cycles of compression to a maximum force of 20 N. Preloading helps to remove any artifacts due to minor asperities on the specimen surfaces. Compression tests were then performed at a strain rate of 0.001 s^{-1} until failure. During testing, force was measured using a 1 kN load cell, and specimen deformation was imaged using a digital camera (Canon EOS, Canon Inc., Japan). After testing, successive images were processed to derive compressive axial strain using custom-written digital image correlation post-processing software [13]. Using the apparent stress *vs.* strain data thus derived, the apparent level Young's modulus was identified by least-squares fitting of the middle third of the linear region of the apparent stress *vs.* strain curve. The ultimate stress was identified as the maximal nominal stress obtained during each test. Note that when reporting mechanical test results, we use the terms 'apparent' elastic modulus and 'apparent' ultimate strength, to refer to the fact that the scale being tested is above that of the tissue level (as would be interrogated by nanoindentation), therefore the apparent properties are a function of both tissue material properties and microstructure.

Resin embedding

Prior to specimen preparation for microscopy, all specimens in both tested and untested groups were fixed in 10% phosphate buffered formalin (Electron Microscopy Sciences, USA) at room temperature for 24 h. Specimens were placed under vacuum at −300 mbar in order to assist formalin penetration. During fixation, the volume ratio of fixative to tissue was kept at or above 10:1. After fixation, all specimens were rinsed under running tap water for 1 h. Specimens were then dehydrated in increasing concentrations of acetone (50%; 75%; 100%), at 24 h per step based on the specimen size [15]. Specimens were then embedded in epoxy resin (Spurr Low Viscosity Embedding Media, Electron Microscopy Sciences, USA) using the corrected formulation of Ellis [16]. Resin impregnated specimens were mounted by placing them inside small rings of plastic which had been pre-glued onto standard 25 × 75 mm microscopic slides, and then filling the space around the specimen with resin. After placement, specimens were degassed under vacuum for 15 min at −300 mbar, and were then polymerized overnight at 60 °C.

Computer numerically controlled milling

After embedding in epoxy resin, a custom built computer numerically controlled (CNC) milling system based on a Proxxon MF70 milling machine (Proxxon Ltd, Germany) was used to mill the mounted OI bone specimens down to the desired thickness for transmitted polarized light microscopy using natural birefringence (200–300 μm). A 3 mm diameter, 4-flute milling bit was used in all milling processes in order to achieve surface finishes in the order of 5–10 μm, which is less than the depth of field of the microscope at the magnifications used for imaging bone microstructure. This system allows preparation of large sections of mineralized bone at thicknesses of several hundred microns, suitable for polarized light imaging of interference colors in unstained specimens.

Polarized light microscopy and image analysis

Since structured arrays of collagen molecules are birefringent, collagen fibers in bone generate interference colors between crossed polarizers, with the color (or intensity in a single wavelength illumination system) containing information about specimen retardation, which can in turn be related to collagen orientation. Although we note that previous studies have quantitatively linked polarization colors to collagen orientation in bone [17,18], in this study polarized light imaging was used qualitatively to provide an indication of the degree of organization of collagen in the bone, and quantitatively in the sense of providing unstained contrast between bone and background for the measurement of intra-cortical porosity.