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Non-osteoporotic women with low-trauma fracture present altered birefringence in cortical bone

Maria-Grazia Ascenzi [a,*], Jesse Chin [a], Joan Lappe [b], Robert Recker [c]

[a] Department of Orthopaedic Surgery, University of California, Los Angeles, USA
[b] Department of Medicine, Creighton University, Omaha, USA
[c] Department of Endocrinology, Creighton University, Omaha, USA

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ABSTRACT

Areal bone mineral density (BMD) by DXA, although an important index, does not accurately assess risk of fragility fracture. Another bone structural parameter, the orientation of type I collagen, is known to add to risk determination, independently of BMD. Accordingly, we investigated the Haversian systems of transiliac crest biopsies from non-osteoporotic women with low-trauma fractures, matched to healthy women without fracture by age and BMD. We employed circularly polarized light (CPL) microscopy because 1) each of the extinct and bright birefringent signals of CPL corresponds to a specific collagen arrangement; and 2) CPL can employ magnification suitable to provide data, of manageable size, from the whole cortical component of a section of biopsy. Under CPL, the coaxial layers of osteons, called lamellae, appear either birefringent extinct or bright. On a section transverse to the Haversian system, the extinct lamella comprises mainly collagen forming small angles, and the bright lamella comprises mainly collagen forming large angles, relative to the general orientation of the Haversian system. We performed semi-automatic morphometry for birefringent and structural parameters for which we computed intra- and inter-observer errors. The statistical analysis used a linear mixed model to compare fracturing and non-fracturing groups while addressing pairing of fracturing and non-fracturing subjects, and linear regression to assess differences between matched subjects. We found significant reduction in 1) lamellar width and area for extinct lamella and bright lamella; 2) percentage of extinct birefringence in osteons, and 3) single osteon area; in the fracturing group; and in lamellar width in the fracturing subject of all pairs. Our results evidence the need to investigate, in a larger sample of subjects, the distribution of collagen orientation as a parameter diagnostic of increased fracture risk.

1. Introduction

Areal bone mineral density (aBMD by DXA), although an important index, does not alone adequately predict bone strength, or risk of fracture. "Compromised bone strength predisposing to an increased risk of fracture" defines osteoporosis, afflicting about 1.5 million people in the US with high morbidity, mortality and costs [1–3]. The clinical and research communities have been seeking factors, that when added to BMD, improve assessment of fracture risk.

Circularly polarized light (CPL) creates extinct and bright signals on bone at the tissue level that correspond to specific orientations of collagen type I (Fig. 1). Collagen type I orientation is a microstructural component of cortical bone, independent of the degree of mineralization, and a predictor of human bone strength ex vivo [4–8] and of animal bone strength ex vivo [9–11]. The effect of orientation of components on bone biomechanics at the tissue level is based on the formation of carbonated hydroxyapatite at the gaps of collagen's staggered pattern (Fig. 1e) that continues on to form elongated crystals extended between, and in the direction of, adjacent collagen fibrils. Electron microscopy of intact and demineralized bone specimens shows the parallelism between the fibril of collagen type I and the adjacent apatite crystallites.

The orientation established by collagen type I strengthens bone in the direction of the orientation under tension, as a two-fiber reinforced composite of collagen and apatite, whose material properties depend on the shared orientation and on the amount of each component [4,5,7,12,13]. Collagen orientation observed in bone at the tissue level varies from location to location and affects bone strength, starting at tissue and microstructural levels, which differs up to 7-fold between regions where the overall collagen orientation differs by 45°–60°, independent of degree of mineralization and loading conditions. Under CPL, the extinct signal corresponds to collagen forming small angles with the axis of the microscope, and the bright signal corresponds to collagen forming large angles with the axis of the microscope. Further, CPL can employ a magnification suitable to provide data of manageable size collected from a whole

* Corresponding author at: Department of Orthopedic Surgery, Rehabilitation Bldg, Room 22-69, 1000, Veteran Avenue, University of California, Los Angeles, CA 90095, USA.
E-mail addresses: mgascenzi@mednet.ucla.edu (M.-G. Ascenzi), jessewchin@gmail.com (J. Chin), jmlappe@creighton.edu (J. Lappe), rrecker@creighton.edu (R. Recker).

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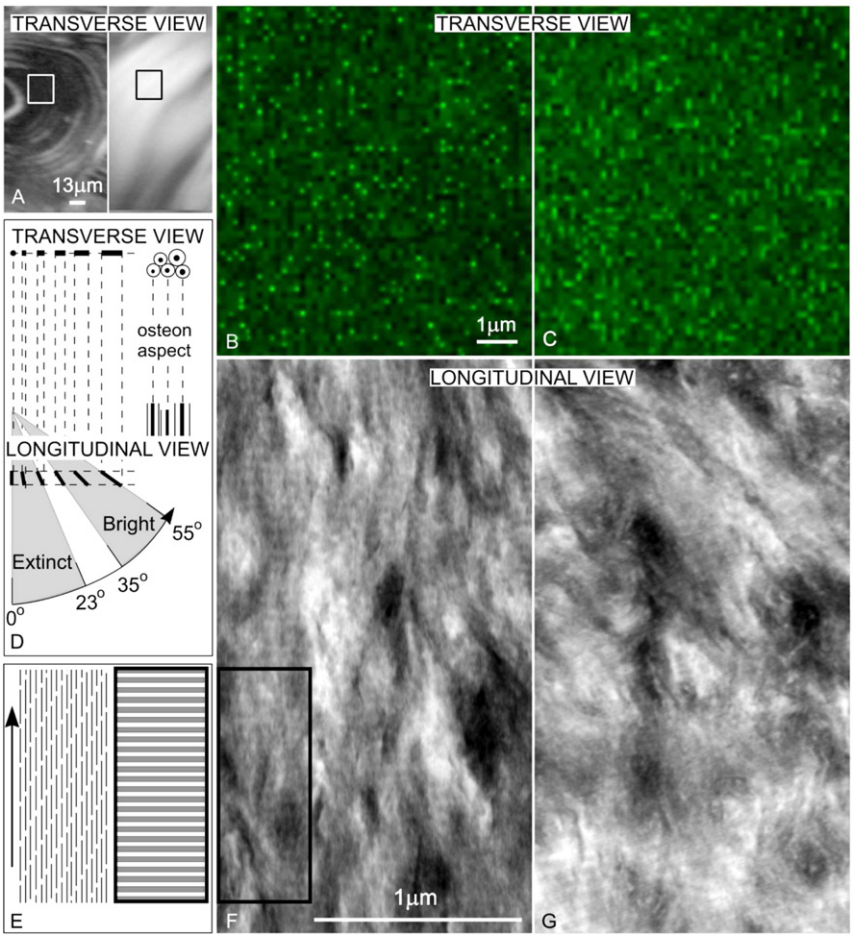

Fig. 1. Extinct and bright signal by CPL: underlying collagen/apatite orientation. (A) Regions (rectangles) of extinct and bright birefringence by CPL in transverse sections are investigated by scanning confocal microscopy (SCM), corresponding to (B) extinct, and corresponding to (C) bright, birefringence. Dots and short auto-fluorescent collagen are more numerous in extinct regions, while longer bundles are more numerous in bright regions. The diagram (D) explains the relationship between appearance of collagen bundles for extinct and bright regions imaged by SCM (B, C) on transverse sections (where the osteon aspect is either circular or elliptical). The diagram (E) explains the arrangement of collagen and its "gaps" where crystallites begin to form. This pattern is viewed in imaging by scanning transmission electron microscopy (STEM) (F, G) on longitudinal sections, cut along the general orientation of the Haversian canals, which defines the longitudinal direction. (F) STEM image corresponding to extinct birefringent transverse region shows a longitudinal collagen pattern. (G) STEM image corresponding to the bright birefringent transverse region shows collagen forming larger angles with the longitudinal direction (from [8] with permission).

cortical component of a biopsy section. For these reasons, we employ CPL for the novel comparison between fracturing and non-fracturing subjects, of the distribution of collagen type I orientation in the iliac crest biopsy.

Our study concerns the cortical component of bone, the contributions of which to bone strength in individuals are increasingly recognized [14–16]. Our previous studies show that collagen type I orientation is improved in a situation where fracture risk is reduced [8]. Indeed, in biopsy sections transverse to the general orientation of the Haversian system, we found that the cortical component maintains the Haversian organization and presents an increased heterogeneity of collagen type I orientation relative to the Haversian system in osteoporotic women treated with teriparatide (hPTH1-34) and hormone replacement therapy. Further, Power et al. found that the width of the lamellae that appear bright on sections transverse to the femoral neck, at the inferior region of the femoral neck, is reduced in fracturing individuals [17].

The hypothesis is that the distribution of the orientation of collagen type I contributes to the quality of compact bone. Specifically, we expected that a woman who fractures, especially a subject with non-osteoporotic levels of BMD, presents an altered orientation of collagen type I with respect to a specified axis of reference. Our line of study probes the orientation of collagen type I as a potential new target for assessment and clinical interventions to reduce fracture risk and to prevent fracture. We intend to investigate whether the orientation of collagen type I is a suitable structural candidate to aid in differentiating bone quality between fracturing and non-fracturing individuals, starting at tissue-level.

2. Materials and methods

2.1. Subjects' specifications

We employed a cross-sectional case–control design to characterize defects in the cortical microstructure of bone underlying low-trauma fractures in postmenopausal women with non-osteoporotic BMD values. A low-trauma (also known as, fragility) fracture is here defined as a fracture occurring from trauma less than a fall from a standing height, excluding fracture of digits, face and skull. We classified each of the reported falls after obtaining detailed descriptions from the study participants, recruited and enrolled at the Creighton University Osteoporosis Research Center after signing an informed consent. The Creighton University Institutional Review Board approved the study. While the most common sites of fragility fractures are spine, hip and forearm, our a priori definition of fragility fracture for this study fits with a definition frequently used in the literature [18,19]. Our investigation involves a subset of 8 pairs of postmenopausal women randomly selected from 60 pairs of women aged 51 to 70 years who had