Review Article

Should Bone Densitometry Define Osteoporosis in 2020? A Current Concepts Review of the Role of Vibrational Spectroscopy in the Evaluation of Bone Health

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Abstract

Bone mineral density (BMD) is the most widely used parameter for measuring bone strength. Indeed, the World Health Organization definition of osteoporosis is based solely on the BMD as measured by dual energy X-ray absorptiometry (DEXA). As our understanding of the factors contributing to bone strength has improved in recent years, this might need to be re-visited. In this review, we have outlined the recent advances in our understanding of the structural health of the bone, specifically how whole bone geometry, micro-architecture and tissue properties are all factors that determine bone strength. We have outlined the importance of micro-crack formation and the pathways that could result following micro-crack formation. We have also presented evidence that makes a case for seeking an alternative technique to DEXA that could potentially improve/augment our ability to assess osteoporosis. Vibrational spectroscopic techniques such as Raman spectroscopy and Fourier transform infrared spectroscopy are evolving as important modalities that have the capability to evaluate all the determinants of bone strength qualitatively and quantitatively in a spatially resolved manner that could potentially provide a much more accurate assessment of bone health.

Keywords: Bone mineral density, dual energy X-ray absorptiometry, fragility fractures, osteoporosis, vibrational spectroscopy

Introduction

Osteoporosis is defined by the National Osteoporosis Foundation as a chronic, progressive disease characterized by low bone mass, microarchitecture deterioration of bone tissue, bone fragility, and a consequent increase in fracture risk.[1] Although this comprehensive definition includes microarchitecture deterioration, the operational definition of osteoporosis is based entirely on the bone mineral density (BMD) measurement. The World Health Organization (WHO) in 1994, defined osteoporosis as a BMD that lies 2.5 standard deviations (SDs) or more below the average value for young healthy women (a T-score of <−2.5 SD).[2] The most widely used method to measure the BMD is dual energy X-ray absorptiometry (DEXA). Its advantages include low cost, low radiation, and patient convenience. Subsequently, it became increasingly obvious that a majority of fragility fractures indeed occurred in individuals whose BMD was not below the osteoporotic threshold of a T-score <−2.5 SD.[3-7] In the 25 years, since the WHO definition was proposed and widely adapted, our understanding of the bone micro-structure and how it deteriorates in osteoporosis, as well as the underlying pathological process involved, has progressed considerably. Our ability to detect these changes has also evolved. This review aims to highlight these advances and let the reader answer the question posed in the title.

Bio-Mechanical Considerations

Loading of Bone Results in Stress. The bone responds by dissipating the stress as well as by resisting deformation up to a certain limit known as the yield point. Beyond the yield

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point, strain starts to develop and could ultimately result in structural failure. The maximal load that can be applied to the bone before structural failure occurs is defined as the strength of the bone. The factors that determine the bone strength are illustrated in Figure 1 and are discussed below.

The mechanical performance of a composite structure like bone is not only reliant on the material properties of its components but also on the manner in which the material is laid out, or in other words, its architecture. The architecture could be conceptualized as macro-architecture or whole bone geometry and the microarchitecture. As the bone is a living tissue, the biological properties determine the dynamic responses to mechanical challenges and thus are central to the viability of the bone. Thus, it is important to consider all the above determinants of bone strength in order to assess the mechanical performance of the bone in health and disease.

Determinants of Bone Strength

Whole bone geometry

The bone geometry contributes to bone strength,[8] and fragility fractures.[9] An increase in bone diameter leads to an exponential increase in resistance to bending and torsion independent of bone mass.[10] Studies have shown that differences in the shape of the proximal femora, such as an increase in the length of the femoral neck and neck-shaft angle, are independent variables associated with an increased risk of sustaining a femoral neck fracture.[11-13] Increased length of the femoral neck leads to an increased moment resulting in a higher concentration of forces in the femoral neck if the person falls sideways.[14] Increased cross-sectional area (CSA) is associated with an increased bone strength index independent of BMD.[15] Indeed, it has been shown that differences in the bone strength between African and Caucasian postmenopausal women[16,17] as well as elderly men and women[18,19] are attributable to differences in the CSA of the bone. Further, cortical thickness but not cortical BMD was found to correlate significantly with the risk of developing fractures.[20]

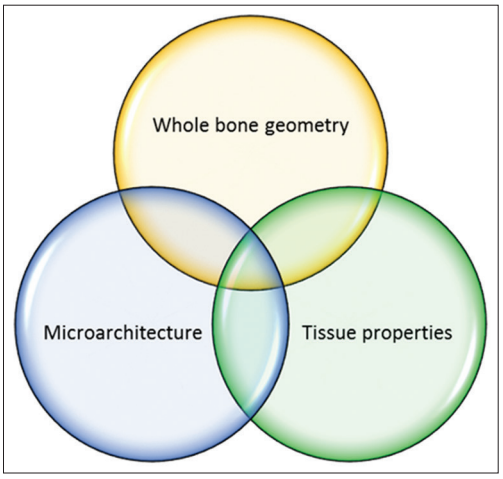

Figure 1: Determinants of bone strength

Microarchitecture

Microarchitectural deterioration of cortical as well as trabecular bone leads to a significant diminution in the bone strength. Due to a larger total surface area relative to volume, trabecular bone is predominantly affected when there is increased bone resorption in osteoporosis. Trabecular microarchitecture is known to vary within the same bone and has been shown to be more relevant than BMD with respect to the site of fracture and the load to failure during compression testing.[21] Trabecular shape (plate-like or rod-like) and thickness significantly influences bone strength, and constitute an independent variable that determines bone strength.[22] Loss of continuity of the trabeculae results from the perforation of individual trabeculae. These changes characterize microarchitecture deterioration.[23-25] Regions such as long-bone metaphyses, and vertebral bodies that have a higher proportion of cancellous bone are affected disproportionately in this process,[26,27] leading to bone fragility in these areas. Females predominantly show a decrease in trabecular number and hence a corresponding increase in trabecular separation, while in the case of males, the prominent feature is a decrease in trabecular thickness.[28] In areas such as the shaft of long bones where cortical bone constitutes a major proportion of the total bone mass, structural deterioration of the cortical bone, if present, significantly contributes to bone fragility. Osteoclasts present in the Haversian channel network, when activated, can cause widening of the channels and hence increased porosity resulting in loss of bone strength.[29,30] An increase in cortical porosity has been shown to be associated with aging independent of the BMD.[31]

Tissue properties

The bone tissue is a two-phase composite with an elastic component mainly composed of type I collagen and a mineral component largely in the form of hydroxyapatite. Type I collagen is produced by osteoblasts initially as a precursor molecule procollagen that subsequently undergoes Post-translational modification in the extracellular matrix and formation of intermolecular and intra-fibrillar cross-links[32] that maintain the closely organized fibrillar structure of collagen and contribute to its tensile strength. The newly formed collagen molecule provides a platform for initial mineralization (primary mineralization), which gradually progresses in terms of the number and size of the crystals (secondary mineralization).[33]

The bone tissue properties and their relevance in osteoporosis can be best understood in the context of the response of bone tissue to loading and microcrack formation/propagation [Figure 2]. Microcrack formation is known to trigger osteocyte apoptosis,[34,35] and the resultant relaxation of the inhibitory control over the osteoclasts[36] as well as the release of stimulatory factors as well as a release of inhibition which in turn leads to osteoclast activation by a combination of loss of constitutive inhibition of osteocytes over osteoclasts and from the release of stimulatory substances.[34,37] These events result in an increase in remodeling locally.[38] In addition to the

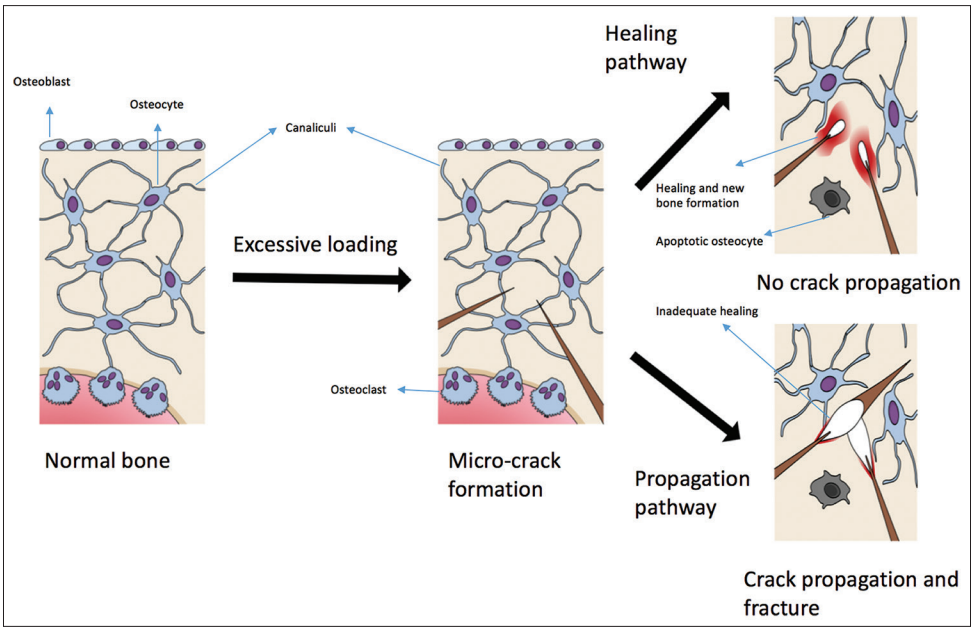

Figure 2: Excessive loading leads to microcrack formation. Following microcrack formation, there are two possible pathways, healing, or propagation. The healing pathway leads to new bone formation and the stoppage of the microcrack. The propagation pathway leads to widening and fracture

above, osteocytes also recruit osteoblasts in response to loading by sensing the hydrostatic pressure of the interstitial fluid as well as detecting cell strain.[39,40] And by chemical signaling involving nitric oxide,[41] Prostaglandins,[42,43] and Sclerostin[44] that modulate osteoblast activity. Beyond a certain capacity to withstand the strain, the bone starts failing structurally, initially by the microcrack formation and if the amount of load keeps increasing, and there is insufficient capacity or time to repair the microcracks, by the propagation of the microcracks and resulting catastrophic failure, which clinically manifests as a fracture.

It is obvious from the foregoing account of tissue properties that they play an important role in the way bone reacts to loads, normal and abnormal. It is also obvious that bulk properties such as the bone mass or bone density do not represent the complete picture in terms of the structural strength and performance of the bone. Consequently, investigations such as DEXA or even quantitative computed tomography (qCT) are not reliable diagnostic or prognostic indicators. This is borne out by several studies, for example, the reduction in bone strength associated with aging is much steeper than the corresponding reduction in BMD.[45] A significant increase in BMD as a result of sodium fluoride therapy was shown to result in an increased rather than a decreased incidence of fragility fractures.[46,47] Improvements in bone strength following exercise have been shown to be independent of BMD in several studies.[48-51] Thus, there is a need to explore a diagnostic modality that could provide more information about the determinants of bone tissue properties in order to diagnose osteoporosis. If it is shown to provide a more accurate assessment of the structural strength of the bone, before gross bone destruction takes place, it could pave the way for early diagnosis and hence, better preventive/regenerative strategies to be adapted. Vibrational spectroscopic techniques such as Raman spectroscopy (RS) and Fourier transform infrared spectroscopy (FTIR) are very promising techniques that deserve emphasis. Their ability to capture tissue heterogeneity at a microscopic level is very helpful because it is well known that even within the same bone, there is a mosaic of pockets of bone resorption and bone formation as well as dormant areas and a balance between them often dictates the mechanical performance of the bone.[52]

Vibrational spectroscopic techniques

Both Raman and FTIR spectroscopy depend on the transition of vibrational energy states of molecules. Infrared spectra are derived directly from the absorption of energy in the infrared range, whereas Raman spectra arise from a scattering of visible or ultra-violet photons. In addition, the biomolecular milieu surrounding the molecule of interest also influence the vibrational pattern and hence can also be analyzed.[53] The pattern of an individual vibrational band is unique for a particular functional group or molecular species.[54] The Vibrational spectral patterns are, for the most part, wavelength-specific; thus, a combination of Raman and FTIR can provide complementary information regarding the test sample [Figure 3].[55] We have previously studied changes in mineralization and collagen structure in human bone using RS.[56] Water does not interfere with this technique and hence it could potentially be used to monitor tissues *in vivo*, noninvasively. The other major advantage is that the tissues can be studied in their native state without any preparations such as contrast media or dyes.

The most useful and commonly assessed spectroscopic parameters are the following:

1. Mineral to matrix ratio: This is the most commonly

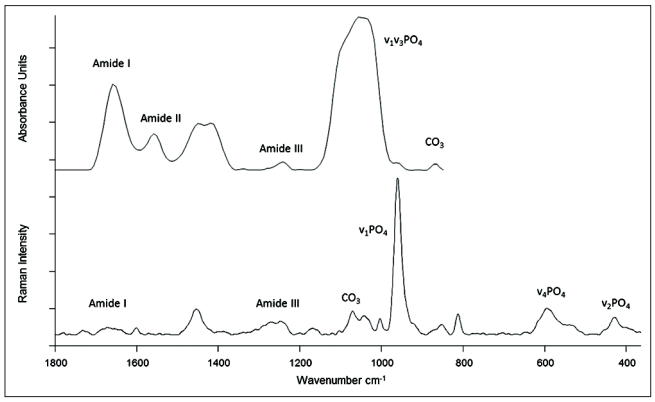

Figure 3: Typical Fourier transform infrared spectroscopy (top) and Raman spectra (bottom) with the commonly studied peaks appropriately marked. Wavenumber is the typical unit of frequency used in vibrational spectroscopy measured in reciprocal centimeters

assessed parameter and has been validated against mineral content measurement by quantitative back-scatter electronic imaging in human bones.[57] It is based on the principle that the integrated area of a band is directly proportional to the concentration of the specific molecular moiety giving rise to it. Most commonly, the ratio of phosphate to amide bands is measured. This is a spectroscopic equivalent of BMD with the added benefit of providing information on the organic matrix (collagen) in a spatially distributed manner

2. Mineral maturity/crystallinity: The chemical makeup of the mineral crystals and their similarity to pure hydroxyapatite crystals is known as crystal maturity whereas the size and the shape of the crystals are referred to as crystallinity. This parameter is highly dependent on the person's age as well as the tissue age in the same person. Heterogeneity of crystals is seen in young healthy bone[58] whereas homogeneity as well as the presence of large crystals is often associated with ageing[59] and osteoporosis[60]
3. Carbonate to phosphate ratio: Bone mineral consists of highly substituted apatite crystals. Carbonate is one of the most abundant substitutions. In healthy bone, the average carbonate is about 6% dry weight.[61] It is most commonly reported as carbonate to phosphate ratio although some report it as carbonate to organic matrix ratio. Carbonate content is known to be altered in osteoporosis[62,63]
4. Relative tissue water content: The contribution of water to the biomechanical properties of the bone is well established.[64,65] Water can be directly measured through quantification of hydroxyl groups by RS[66]
5. Collagen cross-linking: About 90% of the entire organic component of the bone is composed of type I collagen which is a large fibrous protein made up of a triple helix (two $\alpha 1$ and one $\alpha 2$ chains). The most distinctive feature of mineralizing collagen in bone is its cross-linking chemistry and the way the molecule is packed,[67] and contributes significantly to the mechanical properties such as tensile strength and viscoelasticity. The analysis of amide I band can be used to study pyridinoline cross-links and is the parameter most often used to study the cross-link chemistry.[68] It is also the most sensitive spectrometric parameter that differentiates ageing from osteoporotic bone[69]
6. Relative proteoglycan content: Proteoglycans are large noncollagenous glycoproteins. In the bone, they fulfill several important roles such as organic matrix assembly and modulation of mineralization as well as remodeling.[70] They also help maintain unhindered flow of the interstitial fluid through the peri-lacunar/canalicular space of the compact bone by preventing its mineralization.[71] RS can be used to study glycosaminoglycan component of proteoglycans in the bone[72] and a decrease of the same has been reported in postmenopausal osteoporosis.[69]

Several challenges have to be overcome in order to make these techniques widely acceptable as useful clinical tools. With RS, the main challenges have been (a) low signal to noise ratio, (b) background fluorescence, and (c) difficulty in assessing deeper structures as it is largely a surface analytic technique. Various technological advances have largely overcome the signal to noise ratio problem.[73] Background fluorescence problem can be minimized by having a long excitation wavelength[74] and using data evaluation techniques such as Band Target Entropy Minimization technique.[75] Spatially off-set RS,[76] and picosecond time-resolved spectroscopy[77] have made it possible to use this technique for analysis of deeper structures *in vivo*. The main drawback of FTIR is that it is, as yet, an *in vitro* technique and thus, invasive biopsies are required. These biopsies can be obtained if/when the patient is undergoing a bony surgical procedure such as open reduction and internal fixation of a fracture or arthroplasty. Data obtained from the FTIR could provide useful complementary information which can be extrapolated to Raman data as spectroscopic theory allows this extrapolation.[53] Thus, the usefulness of RS as a clinical investigative tool can be enhanced.

In conclusion, these vibrational spectroscopic techniques could potentially facilitate early diagnosis of structural deterioration of the bone at the tissue level. RS has the potential to do this noninvasively and FTIR in a minimally invasive way, before gross/irreversible structural changes occur in the bone architecture.

The ability of vibrational spectroscopic techniques to assess the collagen as well the mineral components of the bone and their intricate inter-relationship and to do so in a spatially distributed manner give these techniques a decisive advantage over DEXA scanning. Drawbacks such as poor signal to noise ratio, need to obtain a biopsy, and portability of the equipment have been largely overcome and the time is ripe for vibrational spectroscopic techniques to move from the bench to the clinic. We recommend that large scale clinical trials evaluating the usefulness of these techniques vis-à-vis established techniques such as DEXA and qCT be conducted. Such efforts would

conceivably go a long way toward assessment and monitoring of bone health.

Ethical consideration

Ethics: As this is a review no ethical committee permission was obtained.

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Conflicts of interest

There are no conflicts of interest.

Authors' contribution

MK conceived the project and was involved with the literature review and writing the manuscript. FK helped with the writing of the manuscript and formatting the references. MSB and JF were involved with the literature search and helped write the manuscript. All authors have reviewed the manuscript, agree with the contents, and take full responsibility for the accuracy of the information contained.

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