

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Bone Mineral Quality

Delphine Farlay^{1,2} and Georges Boivin^{1,2}

¹INSERM, UMR 1033, F-69372 Lyon,

²Université de Lyon, F-69008 Lyon,
France

1. Introduction

The main function of bone is to promote locomotion and protection of vital organs. Bone is also an important mineral ions reservoir, essential to maintain phosphocalcic homeostasis. Bone mineral is a calcium phosphate named "apatite", which form naturally in the Earth's crust (Wopenka & Pasteris 2005). Compared to others minerals, apatite is more "tolerant" and is very accommodating to chemical substitutions. This ability to easily absorb ions confers to bone a detoxification property, with some ions normally absent of bone and which are captured by bone mineral. But the substitutions in bone mineral change the structure of apatite, conferring to bone several properties such as solubility, morphology, hardness, strain etc.

Thanks to those remarkable properties, bone has the ability to continually adapt to changes to its mechanical environment (Bouxsein 2005). Bone is an anisotropic composite material tissue, and highly hierarchical viscoelastic (Bouxsein 2005). When a load is applied to bone, this produces energy, and as this energy can not be destroy, the bone has to absorbed it (Seeman & Delmas 2006). The elastic properties of bone allow to absorb this energy by deforming reversibly. But if the load exceeds the ability of the bone to carry this load, it can deform permanently by plastic deformation (Fig.1). This produces microcracks allowing

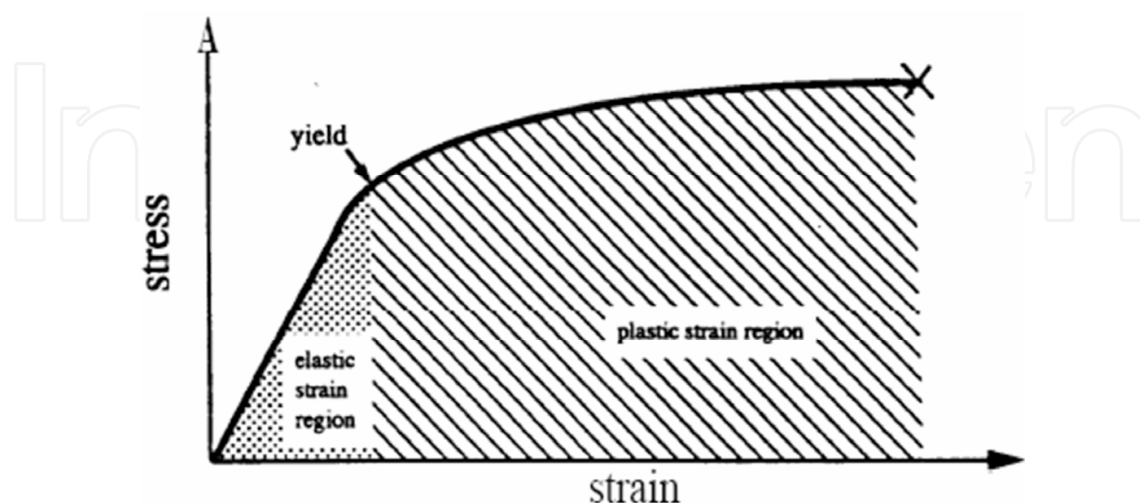


Fig. 1. Stress-strain curve divided into the elastic and plastic regions. The fracture occurs at the end of the curve (marked with X). Reprinted from Turner & Burr, 1993, with permission from Elsevier.

energy release. If the microcracks remain small, this has no impact on bone. However, if the microcracks become numerous and/or too long, the bone fractures. Thus, to resist to a fracture, the bone need to find the best compromise between *stiffness* and *flexibility* (to resist deformation) (Seeman & Delmas 2006). A high mineral content increases *stiffness* reducing *flexibility*, and if the bone is too flexible, it will deform beyond its peak strain and crack. Several studies showed that mineral part is involved in the elastic properties of bone, whereas organic part is rather involved in the plastic deformation (Bala et al., 2011b; Bouxsein 2005; Currey 2003; Follet et al., 2004).

2. Determinants of bone quality

Bone is constituted by three major components which are the organic matrix ($\approx 30\%$, mainly type I collagen), mineral ($\approx 60\%$, carbonated apatite) and water ($\approx 10\%$). Organic matrix is essentially constituted by $\sim 90\%$ of a network of type I collagen fibrils, and $\sim 10\%$ of non-collagenous proteins. Type I collagen molecules are formed by three polypeptides α chains [2 $\alpha 1(I)$ and 1 $\alpha 2(I)$] forming a tight triple helix structure with a repetition of Gly-X-Y triplets (Myllyharju & Kivirikko 2004). To provide the stability of type I collagen fibrils, several mechanisms of maturation and ageing of bone collagen occurs, including enzymatic collagen cross-linking and non-enzymatic modifications (Saito & Marumo 2010; Viguet-Carrin et al., 2006). In parallel, the organic matrix mineralizes, and the organization of type I collagen network determines the specific arrangement of mineral crystals (Höhling et al., 1990; Riggs et al., 1993). The crystals first grow in length, typically plate-like (Landis, 1995; Roschger et al., 1998), then they become thicker but stay relatively thin (Roschger et al., 1998). Concomitantly, the crystals number increases up to physiologic limits of mineralization (Bala et al., 2010; Boivin et al., 2008; Boivin & Meunier 2002).

The quantity of bone mineral (assimilated to the quantity of total bone) is usually measured by bone mineral density (BMD), using dual X-ray absorptiometry (DXA). However, about one-half of fractures occur in women having a T-Score above the World Health Organization (WHO) diagnosis threshold of osteoporosis (≤ -2.5) (Siris et al., 2004; Sornay-Rendu et al., 2005) suggesting that other factors than bone quantity are involved in the apparition of fractures. These factors, involved in bone quality, are called intrinsic determinants. Both extrinsic determinants (including bone mass, macro/microarchitecture) and intrinsic determinants are involved in bone strength, and are directly dependent of bone remodeling activity (Fig.2).

DXA measurement gives information on bone mineral mass but not on its mineral quality. For example, when fluoride salts was used to treat post menopausal osteoporosis, an increase in bone mineral density was measured by DXA, but the bones of patients treated with fluoride salts were much more brittle than untreated patients. In fact, fluoride ions in bone mineral impaired the bone mineral quality increasing the size of bone (large crystals), and thus reducing the contact area with collagen matrix, despite a higher amount of bone mineral density.

The determinants of bone quality, called "intrinsic determinants", are thus essential in bone strength. Those determinants include mineral quality, collagen quality, and presence of microcracks. A good collagen quality is required to an optimal bone strength. For example, osteogenesis imperfecta, which is an heritable brittle-bone disease, is characterized by a type-I collagen mutation, leading to collagen fibrils abnormally thin, and to an excessive

bone fragility (Rauch & Glorieux 2004). Among these determinants, microcracks are normally present in bone, and permit to dissipate energy when bone is submitted to a load. However, the presence of too long microcracks is not good for bone. Thus, all together those extrinsic and intrinsic determinants are involved in bone strength.

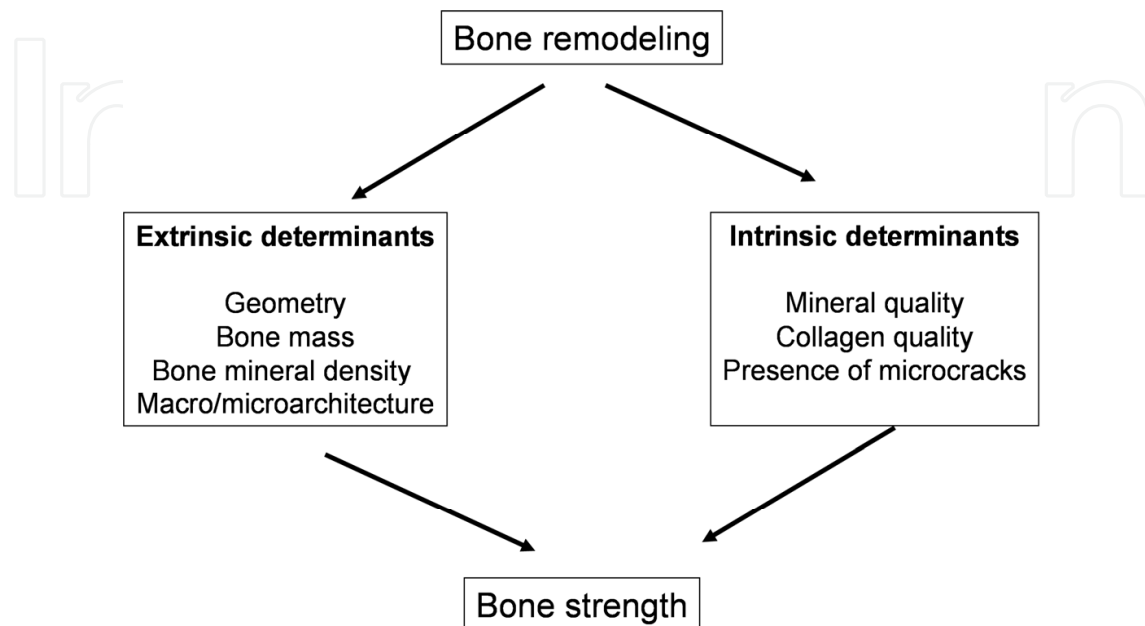


Fig. 2. Description of determinants of bone strength (INSERM UMR 1033)

Bone mineral properties are important determinants of bone strength. Those properties include material (degree of mineralization, hardness...) and crystalline (crystallinity, mineral maturity, ionic substitutions...) characteristics. The importance of the components of bone quality is thus evident and the relationships between the determinants of bone quality are essential to maintain an overall mechanical competent bone. Indeed, bone mineral is complex and knowledge of its composition is important to better understand the mechanisms of bone fragility.

The main purpose of this present chapter is to bring a best approach of bone mineral quality, mineralization process, mineral crystals and mineral composition.

3. Mineralization process: A dynamic process

As bone is submitted to a constant remodeling during all the adult life, old Bone Structural Units (BSUs, named the osteons in cortical bone and the trabecular packets in cancellous bone) will be resorbed by osteoclasts, and replaced by new formed bone. Thus, the recently formed BSUs will be less mineralized than the older BSUs present in interstitial bone and not already resorbed. This heterogeneity of mineralization in the different BSUs can be easily visualized on a X rays microradiograph (Fig. 3). This mineralization process related to bone remodeling can be decomposed into two steps: a primary mineralization which corresponds to a very rapid deposition of first crystals, and a secondary mineralization which is much longer, with a slow and gradual increase in size, perfection and number of crystals.

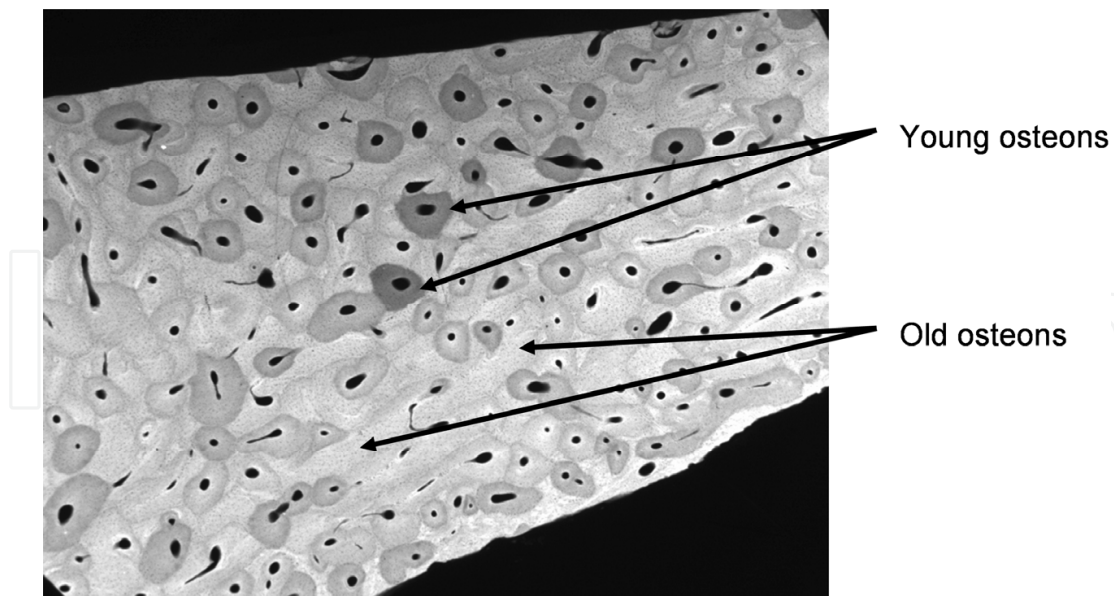


Fig. 3. Microradiograph of human femur illustrating the heterogeneity of the mineralization (cortical bone of a man, 48 year-old) (INSERM UMR 1033)

3.1 Primary and secondary mineralization processes

The process of primary mineralization is a very rapid process, starting in the unmineralized bone matrix (osteoid) deposited by the osteoblasts; in humans, the new matrix begins to mineralize after 5 to 10 days after the deposition of osteoid. The primary mineralization can be measured using double tetracycline labeling (Frost 1969) (Fig. 4). The double labeling involves the administration of two short courses of tetracycline which is deposited along the calcification front as two distinct lines visualized on bone sections under ultraviolet (UV) light. This allows the measurement of the mineral apposition rate (MAR). Usually, a labeling procedure is 10 mg/kg/day demethylchlortetracycline or tetracycline hydrochloride orally for 2 days, 12 days off, 4 days on. The bone biopsy is then taken 4-6 days later (Frost 1969).

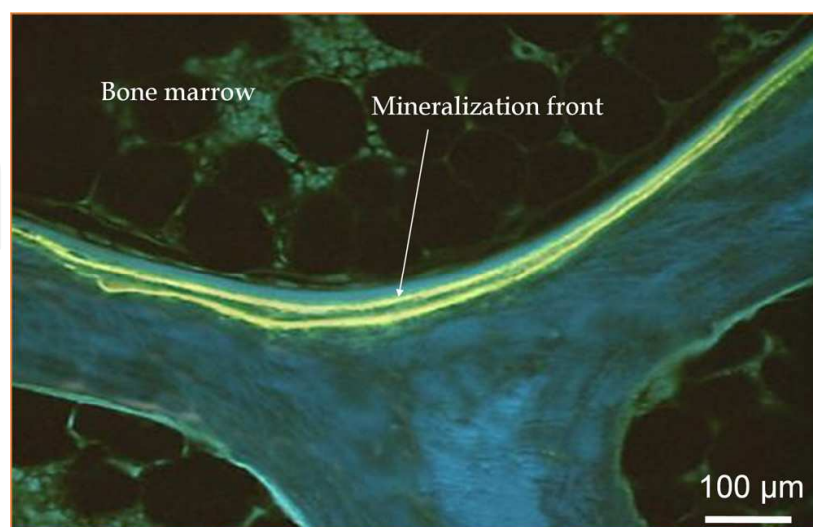


Fig. 4. Histological slide of bone tissue observed under UV light and illustrating a double tetracycline labelling (yellow lines) in trabecular bone and showing the front of mineralization (INSERM UMR 1033).

In adult, MAR varies from 0.60 to 0.80 $\mu\text{m}/\text{day}$ whatever the age and the sex (Vedi et al., 1983). MAR is slightly increased in young children, reaching 1 $\mu\text{m}/\text{day}$ (Glorieux et al., 2000). During the process of primary mineralization, the first depositions of mineral correspond to about 50 to 60 % of the maximal mineral charge in bone tissue (Meunier & Boivin 1997). This process is extremely rapid, and the first depositions of mineral are used as nucleator for the secondary mineralization. The secondary mineralization corresponds to a slow and gradual increase in both crystal size and number. This process increases until a physiological limit: once the maximum number of crystals attained in a given volume, it is not possible to exceed this limit. Thus in bone remodeling, there is no process of “hypermineralization”, because a given BSU can not contain more crystals than its physiological capacity. The duration of the secondary mineralization is unknown in humans. This duration has been reported in rabbits (Fuchs et al., 2008) and more recently in an animal model (ewes) having a remodeling activity close to the Humans (Bala et al., 2010). The chronology of secondary mineralization has been identify by injection of different fluorescent labels every six months, in order to date the “age” of the BSUs (Bala et al., 2010). In this study, it has been shown that the secondary mineralization lasts approximatively for 24 to 30 months, suggesting that after this time, no increase in degree of mineralization occurs (Fig. 5).

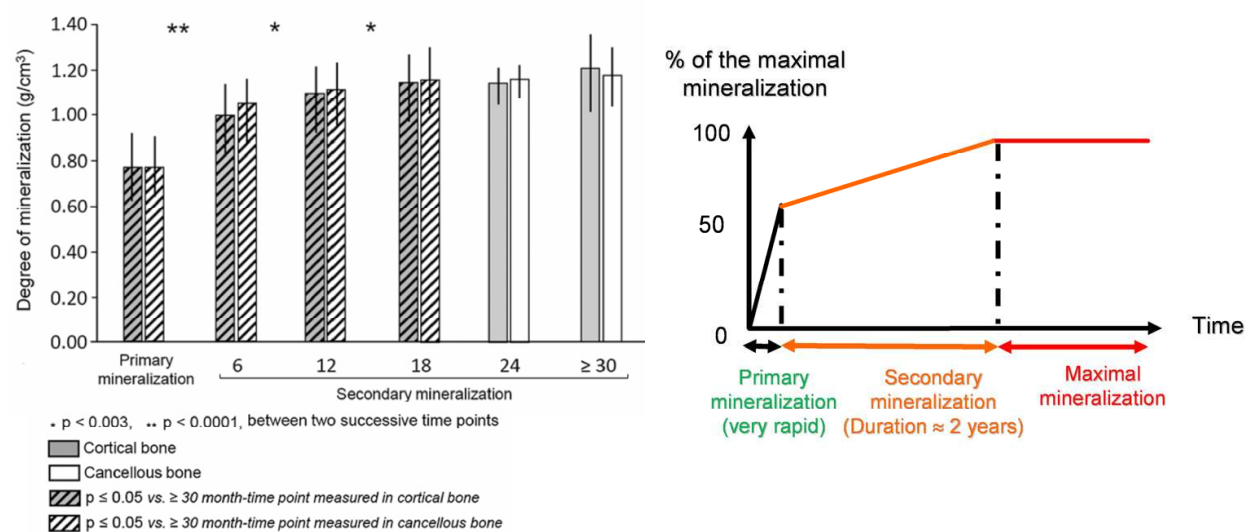


Fig. 5. Left: Degree of mineralization measured by quantitative microradiography in ewes in cortical and cancellous bone, every 6 months (on 512 BSUs; Reprinted from Bala et al., 2010, with permission from Elsevier). Right: Schematic representation of the duration of primary and secondary mineralizations.

This duration of mineralization should be taking into account into anti-resorptive treatment of post menopausal osteoporosis, because once that all the BSUs have attained their maximal mineralization, no gain in term of DMB will occurs.

3.2 Methods to measure degree of mineralization

Several methods are used to measure the degree of mineralization of bone. The technique used in the laboratory is the quantitative microradiography, which is a computerized microdensitometric method based on the X-rays absorption (Boivin & Meunier 2002). Others methods are used to measure the degree of mineralization, as the quantitative backscattering electron or synchrotron infrared microspectroscopy.

3.2.1 Quantitative microradiography

3.2.1.1 Specimen preparation

Undecalcified iliac bone samples were generally used in humans, fixed in 70% alcohol for ten days or more (depending on the size of the samples), and then specimens are placed two days in absolute alcohol to complete dehydration. Alcohol baths are changed every day and specimens are substituted in methylcyclohexane again for two days, before embedding in methyl methacrylate (MMA). The latter is a transparent and hard plastic having a very low X-ray absorption power. Samples are kept for two days in MMA monomer alone, at 4°C, two days in MMA with 1% of catalyst (anhydrous dibenzoyl peroxide) and 2 days in MMA with 2% of catalyst. Then the specimens are placed in an oven (30°C) for final polymerization to obtain hard blocks. After polymerization, thick sections are cut from the embedded bone samples with a precision diamond wire saw, progressively ground to a thickness of 100µm and polished with an alumina suspension. The thickness of the section was measured with an accuracy of 1 µm using a precision micrometer. After ultrasonic cleaning in demineralised water, the bone sections were microradiographed. If orientation of the blocks is possible before sectioning, the cutting plane perpendicular to the haversian canals of cortical bone is preferred.

3.2.1.2 Measurement of degree of mineralization (DMB) (Boivin & Meunier 2002)

Soft X-rays are produced in a X-ray generator (Philips compact PW1830/40 X-ray diffraction generator, Limeil Brévannes, France), equipped with a diffraction tube PW 2273/20. A monochromatic X-ray beam is employed, i.e., nickel-filtered copper K α radiation with a wavelength of 1.54 Å for which the ratio of the mass- absorption coefficients of aluminium to apatite is 0.561. The distance between the X-ray source and the specimen is about 25-30 cm. In a dark room, the 100 µm-thick bone sections are placed on a photographic emulsion covered by a thin polyester (mylar) film transparent to X-rays, and placed in a specimen holder. An aluminium step-wedge is also exposed on each microradiography. Aluminium was chosen because it is convenient material having an atomic number not far from the effective atomic number of hydroxyapatite. The section is firmly pressed flat by tightening the specimen holder cap and evacuating the air situated between the mylar and the emulsion by means of a vacuum pump, thus bringing the section in direct contact with the emulsion. The specimen holder is placed in a camera perpendicular to the X-ray beam and locked into position during X-ray exposure, during 20 min at 25 kV and 25 mA.

After X-ray exposure, the film (VRP-M green sensitive emulsion from Geola, Slavich International Wholesale Office, Vilnius, Lithuania) is developed for 5 min in Kodak D19 at 20°C, rinsed and then fixed for 5 min in Ilford Hypam. The film is washed and dried, then mounted between two slides. The DMB is quantified using an automatic program for analyzing grayness levels (MorphoExpert and Mineralization, ExploraNova, La Rochelle, France). A digital camera (resolution: 1600 x 1200 pixels or 800 x 600 after binning), captures the microscopic image of the microradiograph. After calibration with the aluminium reference system, the measured regions of bone tissue are automatically selected, and the gray levels are segmented after bone thresholding. The values of the gray levels are then obtained at pixel level (for a magnification x2.5, the size of the pixel is 2.82 µm). Finally, gray-level are converted into DMB measurements with the construction of a calibration curve based on the measurements obtained on the aluminium step-wedge. DMB is finally expressed in gram of mineral over cm³ of bone (g/cm³) and measured separately in cortical

and cancellous bone. The main parameters, extracted from the DMB measurements, are the mean DMB, the mean highest and most frequent DMB (DMB Freq. Max) and the mean index of heterogeneity of the distribution of DMB expressed as the mean of the widths at half-maximum measured on the individual DMB curves.

3.2.2 Quantitative backscattering electron imaging (qBEI)

The mineral content of bone samples has also been evaluated (Roschger et al., 1995, 1998, 2003; Ruffoni et al. 2007) by quantitative Backscattered Electron Imaging (qBEI). This method, based on the detection of electrons backscattered (BSE) on the surface of the bone specimen, is generally used on the same type of bone biopsy fixed in alcohol and embedded in MMA. As the intensity of the BSE signal is strongly related to the atomic number (Z) of the specimen, BSE images provide information about the distribution of different elements in the sample. A calibration of the BE signal with carbon and aluminium as references was performed. Osteoid and hydroxyapatite were also employed as references to convert gray level values into calcium weight % values. In bone, the main signal is related to Ca ($Z=20$) and P ($Z=15$) which are the main mineral elements (Roschger et al., 1998). These authors have correlated BE gray levels of bone with calcium content (in weight percent Ca) based on the Ca $K\alpha$ -line intensities detected from identical bone areas (Roschger et al., 1995). The intensity of the backscattered electron signal from the sample is directly proportional to the bone calcium concentration and can therefore be used for the generation of bone mineralization density distribution (BMDD). BMDDs display the frequency of certain calcium concentrations and are analyzed for the weighted mean calcium concentration (Ca mean), the most frequent calcium concentration (Ca peak) and the homogeneity of mineralization (Ca width). The BMDD of trabecular bone from healthy, adult individuals was shown to be nearly constant over several biological factors (gender, age, ethnicity, skeletal site). Technical and biological variations showed that it is a method sensitive for subtle changes in mineralization.

3.2.3 Synchrotron radiation microtomography (SR μ CT)

Aside from the difficulty of access to synchrotron radiation facilities, a main advantage of this technique is the use of a mono-energetic synchrotron beam, thus avoiding beam-hardening effects. Indeed, the reconstructed gray levels of tomographic images correspond directly to a map of a linear attenuation coefficient within the sample. The SR μ CT method has been tested on human bone tissue (Borah et al., 2005, 2006; Nuzzo et al., 2002) but it is still an equipment with difficulty accessible. The availability of a three-dimensional (3D) measuring technique coupled to specific image processing method opens new possibilities. SR μ CT may provide 3D images with spatial resolution as high as one micrometer. The acquisition of 3D bone samples images at high spatial resolution using SR μ CT has proved to be very accurate for quantifying human bone micro-architecture. Moreover SR μ CT is a non destructive, fast, and very precise procedure to determine the DMB in 3D, simultaneously to the micro-architecture. The calibration procedure used homogeneous phantoms of water solutions at different concentrations of K_2HPO_4 (Nuzzo et al., 2002). This method was compared with the quantitative microradiography technique on the same bone samples, and showed that the values of the DMB are both in the range 0.5-1.6 g/cm³ of bone, both in cortical and cancellous bone, with a mean difference around 4.7%, slightly higher in trabecular region (Nuzzo et al., 2002).

3.3 Bone microhardness at the tissue level

Another important characteristic of bone mineral is its hardness (Currey 2003; Nalla et al., 2003). Thanks to indentation techniques, it has been shown that microhardness of bone osteon was strongly related to its mineral content (Amprino 1958; Bala et al., 2010; Boivin et al., 2008; Carlstrom 1954; Weaver 1966). From a mechanical point of view, microhardness parameter is related to both elastic and plastic deformations, and an indentation technique has been developed to measure directly both elastic modulus (E) and contact hardness (H_c) on small area of bone tissue (Oliver & Pharr 1992). However, this technique has been developed for isotropic materials. While it is known that the bone tissue is complex with an anisotropic structure, the measurements of E is usually performed with a defined Poisson's ratio ($\nu=0.3$). It has been shown that contact hardness was linearly interdependent with elastic modulus (Oyen 2006). Therefore, contact hardness can give an evaluation of bone stiffness which is directly related to its brittleness. Contact hardness can be evaluated at the microstructural (BSU) or nanostructural (lamellar) levels. At the microstructural level in bone, the pyramidal square-based Vickers indenter is often used (Fig. 6) (Boivin et al., 2008), and nanoindentation is rather used at the lamellar level using Berkovich indenter (Ammann & Rizzoli 2003). Very recently, it has been shown, by instrumented nanoindentation, that contact hardness was correlated both to DMB and collagen maturity (Bala et al., 2011b). Mineralization is a major determinant of microhardness, with about two-thirds of the variance, and one-third being explained by the organic matrix (Boivin et al., 2008). In human control bone, the microhardness does not vary with age and sex, in cortical and cancellous bone, as for the degree of mineralization. In 19 human control bones, the hardness in cortical bone is about 49.30 ± 2.16 kg/mm² and in cancellous bone about 48.92 ± 1.57 kg/mm².

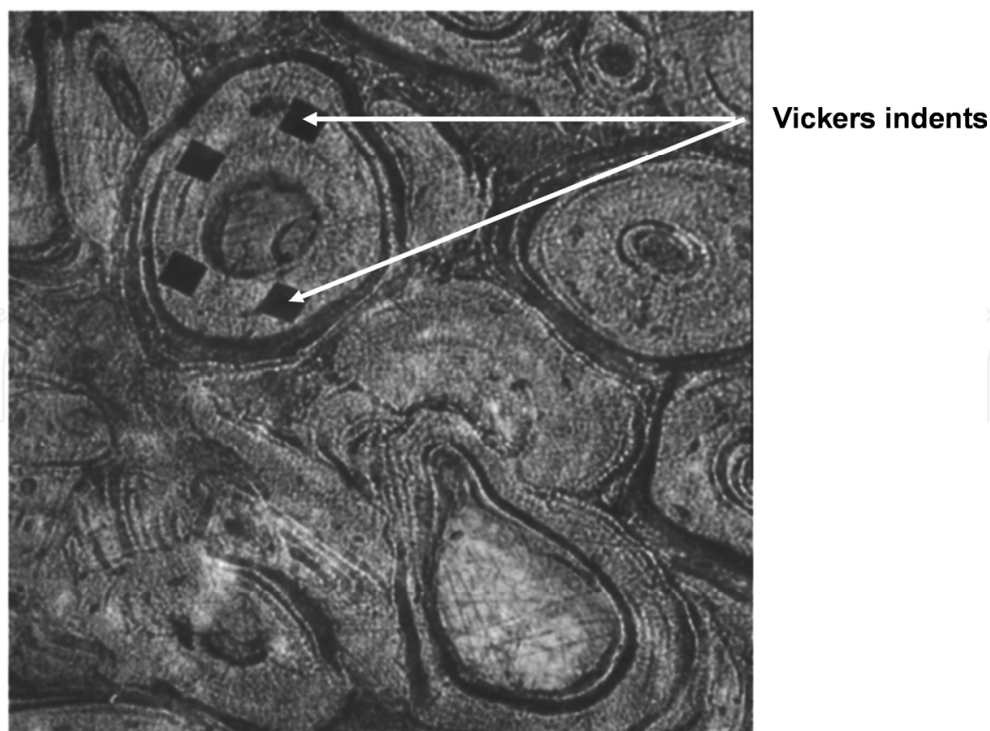


Fig. 6. Iliac bone from ewe showing a Bone Structural Units (BSU) with 4 Vickers indents (INSERM UMR 1033).

4. Characteristics of bone mineral crystals

Human bone mineral is a non-stoichiometric and poorly crystallized apatite. Bone apatite structure is hexagonal with space group $P6_3/m$, with lattice parameters $a=9.42\text{\AA}$ and $c=6.88\text{\AA}$. It is a calcium (Ca)-deficient apatite analog, contains major elements like calcium, $[\text{Ca}^{2+}$ (40 wt %)], phosphate $[\text{PO}_4^{3-}$ (18 wt %)], carbonates $[\text{CO}_3^{2-}$ (6-7 wt %)], minor elements such as magnesium (Mg^{2+}) or sodium (Na^{2+}), and trace elements (LeGeros & LeGeros 1983; LeGeros et al., 1968). Bone mineral also contains ions normally absent from body fluids (lead, fluoride, aluminium etc). Indeed, the apatite lattice is very tolerant to substitutions and vacancies. Compared to dental enamel, the bone mineral contains much more vacancies. In fact, apatite is able to incorporate itself, in its atomic structure, the half of the elements in the periodic chart (Wopenka & Pasteris 2005). Apatite lattice contains about 40 ions, and the unit cell is the smallest basic unit which is a sample of the entire lattice array (Glimcher 1998). In the apatite unit cell, four different types of crystallographic positions (or "sites") have been identified (Fig.7): (1) tetrahedral sites for six P^{5+} -ions, each in 4-fold coordination with oxygen, (2) Ca[I] sites for four of the Ca^{2+} ions, (3) Ca[II] sites for the six other Ca^{2+} ions, and (4) the channel site, occupied by two monovalent anions (OH^- , F^- and/or Cl^-) (Posner 1969; Wopenka & Pasteris 2005). The small ions (Cd^{2+} , Zn^{2+} , Mg^{2+}) are preferentially incorporated into Ca[I], whereas bigger ions (Sr^{2+} , Ba^{2+} , Pb^{2+}) are incorporated into Ca[II] (Fig. 7). The reason why apatite is the mineral component of vertebral skeleton is not known, but it was shown that apatite is the only calcium-phosphate mineral phase that is stable at both a neutral and basic pH (Glimcher 2006; Omelon et al., 2009). Another explanation is coming from the presence of dense granules containing polyphosphates near the mineralizing cartilage and resorbing bone (Omelon et al., 2009). Indeed, when the mineral apatite is dissolved after acidification and resorption by osteoclasts, there is no reprecipitation within the resorption pits, even the return to a neutral pH. The hypothesis of the authors was that polyphosphates formation provides a mechanism for accumulating phosphate, controlling the apatite at locations sites previously mentioned. Enzymatic action can thus control apatite supersaturation at neutral pH, directly by controlling orthophosphate ion activity (Omelon et al., 2009).

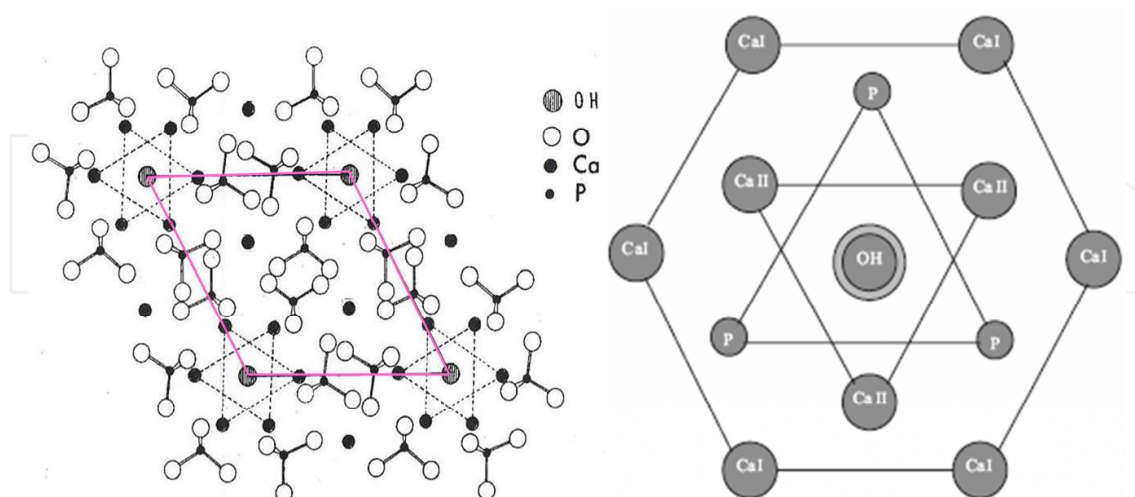


Fig. 7. (Left) Hexagonal system of apatite lattice, showing the disposition of atoms. (Right) The Ca ions occupy two crystallographic non-equivalent sites (Ca I and Ca II). From (Reprinted from Li et al., 2007, with permission from Elsevier). Small ions (Cd^{2+} , Zn^{2+} and Mg^{2+}) are preferentially incorporated into Ca[I], whereas bigger ions (Sr^{2+} , Ba^{2+} and Pb^{2+}) are incorporated into Ca[II].

4.1 Bone crystal size and shape

The crystal structure and morphology of bone minerals have often been controversial, mainly due to the different techniques used to characterize bone mineral. Today, with the use of more accurate method (atomic force microscopy, high resolution transmission electron microscopy), it is clear that the bone mineral crystals are very small and platelet-shaped (length $\approx 200\text{-}600 \text{ \AA}$, width $\approx 100\text{-}200 \text{ \AA}$, thickness $\approx 20\text{-}50 \text{ \AA}$, Figs. 8 and 9). Compared to bone crystals, enamel crystals are needle-shape and much bigger. This small bone crystal size has several advantages. First, it permits an extended surface area ($100\text{-}200 \text{ m}^2/\text{g}$). Two factors are involved in the surface activity: the surface area expressed in m^2/g and the physical and chemical properties of the surface. These properties determine the type of reactions, while the surface area determines the number of reactions. The combination of both factors makes the bone mineral substance metabolically very active; consequently, crystals have a very large interface with extracellular fluids. For example, the crystals contain in a small lumbar vertebra (L1 or L2) having a dry wet of 30 g, have a specific surface comparable to that of the playing field of soccer. Bone mineral is metabolically active, various and numerous interactions between ions from the extracellular fluid and ions constituting apatite crystals, are thus possible. Second, another interest of the small crystal sizes is mechanic. Indeed, the highly ordered location and orientation of very small crystals within the collagen fibrils permits an acceptable range of flexibility without fracture or disruption of the bone substance (Glimcher 1998; Landis 1995).

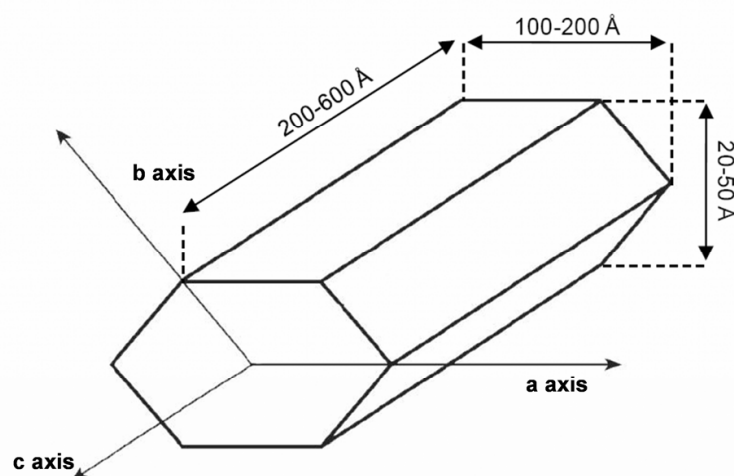


Fig. 8. Schematic representation and crystal size of bone apatite with the 3 axis (INSERM UMR 1033)

4.2 Relationships between water, organic matrix and mineral

In bone, the process of crystal nucleation in bone matrix is heterogenous, and is formed within the "hole" band of type I collagen (670 \AA) (Glimcher et al., 1957). During the process of mineralization, the apatite crystals replaced some of the molecules of water so their content is inversely proportional to that of water (Elliott & Robinson 1957; Robinson 1975). Once deposited, the mineral phase induces compaction of the collagen fibril structure. Neutron and X-ray diffraction have shown that the Bragg-spacing of collagen strongly decreases with increasing mineral content (Lees 1987). Computer modeling and SAXS confirmed the process of closer packing of the collagen molecules when clusters of mineral

crystals replaced the water within the fibrils (Fratzl et al., 1993). The expansion of mineral crystals compressed the collagen molecule packing, thus decreasing the molecular spacing. This indicated the close relationship between water and the mineral deposition process. Modification of the collagen packing probably influences secondary structure of organic matrix. More recently, in intact bovine bone, the effects of dehydration have been studied using solid-state NMR spectroscopy (Zhu et al., 2009). Interestingly, well-resolved peaks broadened with dehydration, suggesting a conformational disorder and structural changes of bone matrix. This is in agreement with other studies showing a collagen conformational change with dehydration (Naito et al, 1994 ; Saito et al, 1984, 1992).

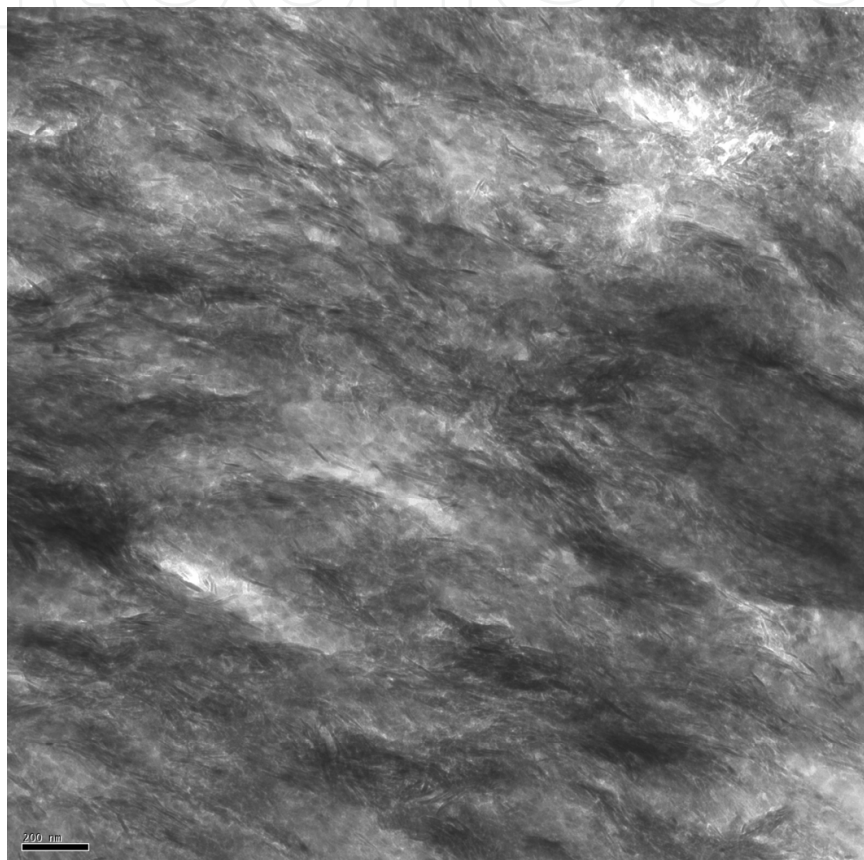


Fig. 9. Electron micrograph of human cancellous bone (woman, 80 year-old) illustrating the bone crystals within the type I collagen (INSERM UMR 1033).

It was suggested that water could play a role in the mechanical behavior of cortical bone (Nyman et al., 2006) and the removal of water alter crystallographic structure of synthetic apatites (LeGeros et al., 1978). As the water content decreases in bone with age (Jonsson et al., 1985; Mueller et al., 1966), it was suggested that water could be involved in bone fragility. Three main types of water exist in bone: the freely mobile water located into vascular lacunar canalicular spaces, the water bound to the collagen network, and the water bound to the mineral. More precisely, there is two type of water bound to the mineral: (a) the water bound to the surface of bone crystals, and (b) the water located within the apatite lattice. Water bound to the collagen fibrils provides post-yield toughness to bone and when water was removed, the strength and stiffness was increased whereas the toughness was decreased (Nyman et al., 2006). The role of the loss of water bound to the mineral on bone

strength was not clear in this study, while it was suggested that the loss of water located in apatite lattice could change the size of the bone mineral crystals (Nyman et al., 2006), since it was already observed in dehydrated enamel or precipitated apatites (LeGeros et al., 1978). Consequently, the decrease in bone strength and toughness related to age could be due to a change in water distribution.

4.3 Chemical composition of bone mineral

The composition of bone mineral was for a long time assimilated to hydroxyapatite, but it is not. Several studies showed the lack of OH⁻, by inelastic neutron scattering, Raman or infrared spectroscopies (Loong et al., 2000; Pasteris et al., 2004; Rey et al., 1995b). Solid state-NMR shows that the percentage of OH⁻ does not exceed 20% of the amount expected in stoichiometric apatite in human cortical bone (Cho et al., 2003). In fact, the small crystal size could be one of the reasons for the absence of OH⁻ ions in the bone apatite. Indeed, Wopenka and Pasteris have suggested that the small crystal size and the great the atomic disorder within the unit cells of the crystal was not energetically favourable for apatite for incorporate OH⁻ into its channel (Pasteris et al., 2004). Another reason for the lack of OH⁻ ions in bone apatite could be due to the type-B substitution of PO₄ by CO₃, creating a vacancy in the channel site to maintain electrostatic equilibrium.

Nature of apatite	Chemical formula
Hydroxyapatite	Ca ₁₀ (PO ₄) ₆ (OH) ₂
Bone mineral	Ca _{8.3□1.7} (PO ₄) _{4.3} (HPO ₄) (CO ₃) _{1.7} (OH) _{0.3□1.7}
Dental enamel	Ca _{9.4□0.6} (PO ₄) _{5.4} (HPO ₄) (CO ₃) _{0.6} (OH) _{1.4□0.6}

Table 1. Chemical formula of different apatites (□: vacancy) (adapted from Cazalbou et al., 2004a)

When an ion with the same electric charge is substituted within the bone apatite, no effect is produced on the structure lattice. If ions have a different electric charge (CO₃²⁻ substituted for PO₄³⁻), a vacancy is created to maintained electrostatic equilibrium. Some ions can be replaced by other ions of almost identical radius. These ions induce only minor changes in shape at crystal level and do not affect the structure of the crystal. Such substitutions occur during formation of the crystal or through ionic exchanges with the existing crystals. In vitro, some interactions between mineral substance and the solution lead to the diffusion of ions within the hydrated shell, the exchanges at the crystal surface and the exchanges inside the crystal. Similar mechanisms are likely to occur in vivo during remodeling of bone tissue (Blumenthal 1990; LeGeros 1981; Posner 1985). Some cations of similar size and charge as the Ca²⁺ (Sr²⁺, Na⁺), as well as others that cannot substitute for the Ca²⁺ in the apatite structure (Ba²⁺, Ra²⁺, Mg²⁺, K⁺), are easily exchangeable from the solution to the surface Ca²⁺ ions. Such substitutions lead to modifications in the *a* and *c* parameters of the apatite unit cell. Substitutions with Mg²⁺ are only partial. In biomimetic apatite nanocrystal, the incorporation of Mg has been recently studied to analyze the effect of substitution with Ca²⁺ on particule morphology (Bertinetti et al., 2009). Incorporation of Mg²⁺ does not affect apatitic nature, nonetheless, a lower degree of crystallinity was observed by XRD (Bertinetti et al., 2009). Moreover, it was shown that apatites enriched with Mg²⁺ retain more water at their surface than Mg²⁺-free apatites (Bertinetti et al., 2009). Calcium can be easily substituted by

significantly larger ions, but less frequently by smaller ions (Blumenthal 1990). An ion can only be substituted to another if its ionic radius is less than 10% higher than the radius of the ion replaced. The exchange of anions (PO_4^{3-} , F^-) around the crystal surface is also well known. The substitution of F^- with OH^- ions can not be reversed because it leads to the formation of a more stable compound: F^- ions are more similar to Ca^{2+} ions than OH^- ions and the electrostatic links between Ca^{2+} and F^- are stronger than the ones between Ca^{2+} and OH^- . The more insoluble apatite is the fluoroapatite [chemical formula: $\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$]. To be resorbed by osteoclasts, the bone mineral have to be more soluble than hydroxyapatite, and the vacancies present in bone mineral enable this dissolution. Carbonates can be found in apatite crystals as CO_3^{2-} ions, which can substituted for either PO_4^{3-} or OH^- ions. When the volume of CO_3^{2-} ions increases, the a parameter of the crystal unit cell decreases, while the c parameter increases.

Some foreign ions can increase or decrease the bone crystal size. In the case of Mg^{2+} , *in vitro* studies have shown that Mg^{2+} bound to the hydroxyapatite crystals retarded nucleation and growth of the crystal. *In vivo* studies show a decrease in crystal size in Mg-deficient rats, thus Mg interferes with the mineralization process (Bigi et al., 1992; Blumenthal et al., 1977; Boskey et al., 1992).

Others ions, as Fe^{3+} ions, have a direct effect on hydroxyapatite, inhibiting the growth and changing the quality of crystals (decrease in crystallinity and increase in carbonate substitution) (Guggenbuhl et al., 2008). Aluminium also affects bone mineralization, and osteomalacia renal osteodystrophy has been associated, in patients on long-term hemodialysis, with Al^{3+} accumulation in bone (Blumenthal & Posner 1984). A recent study on rats showed that a long-term Al^{3+} exposure reduces the levels of mineral and trace elements in bone (Zn, Fe, Cu, Mn, Se, B, and Sr) (Li et al., 2010b). This is accompanied by a decrease in BMD especially in cancellous bone. An high amount of ions which are normally present in small proportion in bone mineral can cause alteration of bone substance. As previously mentioned, fluoride ions at high doses cause osteomalacia and defects of mineralization (Balena et al., 1998). On the other hand, small doses of some ions can have positive effect on bone strength. For example, Sr^{2+} (Strontium ranelate is an osteoporosis treatment) reduces both the vertebral and non vertebral fractures (Meunier et al., 2004; Reginster et al., 2005). Besides the effect of Sr^{2+} on bone cells (stimulating bone formation and decreasing bone resorption) (Grynepas & Marie 1990; Marie et al., 1993), the presence of Sr^{2+} is shown in the bone mineral formed during treatment, in osteoporotic women treated with strontium ranelate for 3-5 years (Boivin et al., 2010; Doublier et al., 2011a, b). The concentration of Sr^{2+} is very low, and do not exceed in human a maximum of 0.5 ions Sr^{2+} for 10 Ca^{2+} (Li et al., 2010a). Moreover, the thickness and length of the plate-shaped bone mineral crystals were not affected by the strontium ranelate treatment (Li et al., 2010a). Presence of Sr^{2+} causes no osteomalacia, no modification in the mineralization process or crystal size. However, the Sr^{2+} increases the bone strength, thus the presence of Sr^{2+} in bone mineral has certainly positive effects, but this mechanism is to date unknown.

4.4 Bone apatite: A particular structure, with a hydrated layer around an apatitic core

The surface of bone crystals, formed in the water of extracellular fluid, exhibits a "hydrated layer" (Fig. 10). Ions in this layer are very labile and reactive, and constitute the non-apatitic domain, surrounding the relatively inert and more stable apatite domain of the bone crystal (Cazalbou et al., 2004; Termine et al., 1973). Newly deposited bone mineral contains many labile non-apatitic domains [HPO_4 , PO_4 , and CO_3], located in the

well-developed hydrated layer involved in the high surface reactivity of mineral (Cazalbou et al., 2004). Labile PO_4 and CO_3 groups are easily and reversibly exchangeable with other ions in the hydrated layer. During maturation, the decrease in labile non-apatitic environments is associated with an increase in stable apatitic environments (Cazalbou et al., 2004). A particularity of the bone mineral is its non-stoichiometry, leading to the presence of numerous vacancies in the apatite crystal. Consequently, bone crystal is mainly maintained by electrostatic cohesion, thus bone crystals are easily soluble relative to stoichiometric apatite (Barry et al., 2002). As bone becomes more mature, both the size and number of crystals increase.

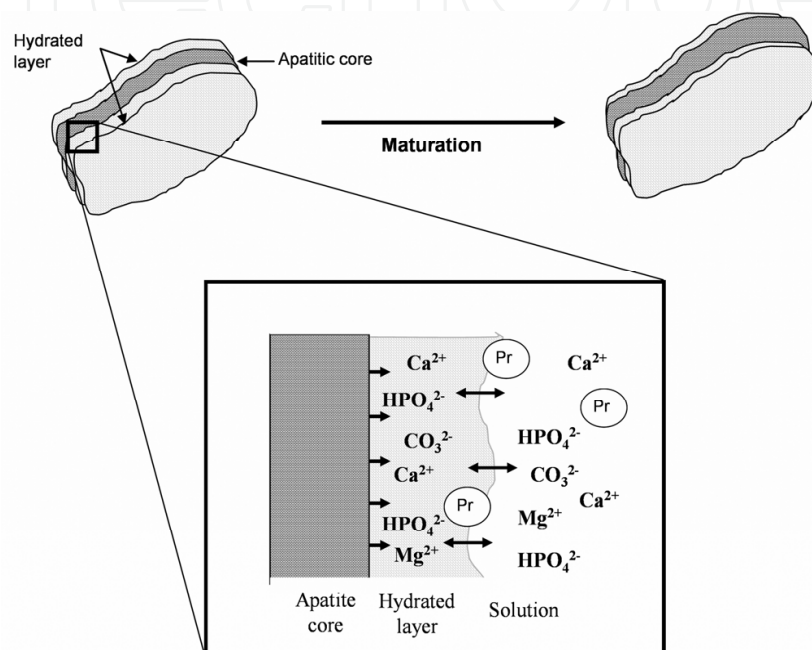


Fig. 10. Evolution of the hydrated layer and apatite core from bone crystal. During the maturation and growth of the crystal, the hydrated layer, involved in a high surface reactivity, progressively decreases and led to a stable apatitic domain. The structure of the hydrated layer constitutes a pool of loosely bound ions which can be incorporated in the growing apatite domains and can be exchanged by foreign ions in the solution and charged groups of proteins (Pr) (Adapted from Rey et al., 2009).

During mineral maturation, the hydrated layer decreases while the stable apatite domain grows, corresponding to the evolution of non-apatitic environments into apatitic environments detected by Fourier Transform InfraRed spectroscopy (FTIR). This hydrated layer, different from a hydration layer (Stern double layer), corresponds to the mode of formation of apatite crystals in physiologic conditions. The existence of these two domains (hydrated layer and apatite core) in biomimetic nanocrystals, has been recently confirmed by solid-state NMR (Jager et al., 2006). The hydrated surface layer contains loosely bound ions, which are easily exchangeable, and determine the surface properties of the nanocrystalline apatites (Cazalbou et al., 2004; Termine et al., 1973). In bone, those loosely bound ions can be also exchanged with charged groups of proteins present in collagen and non-collagenous proteins. The role of charged proteins on mineralization is well known (Boskey et al, 1989, 1998; Boskey 1989; Georges & Veis 2008; Landis et al., 1993; Malaval et al., 2008; Traub et al., 1992).

4.5 Measurement of mineral crystallinity

Crystallinity is defined as the degree of structural order in a crystal. The atoms in the crystal are arranged in a regular and periodic manner. The referent method to measure *absolute crystallinity* is the X-rays diffraction, and is based on the elastic scattering of the X-rays. This technique can be used to determine the crystal structure (Le Bail & Louër 1978; Rietveld 1969) or chemical composition of a sample through the power diffraction bank data file (Powder diffraction file). The WAXS (wide angle X-rays scattering) is based on scattering angles 2θ larger than 5° , and SAXS (small X-ray scattering) gives informations on angles 2θ close to 0° . This method gives informations on the size, strain and orientation of crystals. A peak broadening can be due the small crystal size or microstrains. The crystal size can be determined by the *Scherrer* equation.

Vibrational spectroscopy techniques, such as Fourier Transform InfraRed Spectroscopy (FTIRS), Synchrotron InfraRed or Raman Spectroscopy, have been extensively used to study calcified tissues (Ager et al., 2005; Akkus et al., 2003; Boskey et al., 2005; Cazalbou et al., 2004; Farlay et al., 2010b; LeGeros 1981; Miller et al., 2001; Paschalis et al., 1996; Pleshko et al., 1991; Rey et al., 1990, 1991). Spectroscopic techniques allow assessment of physicochemical modifications of mineral induced by mechanical tests (Ager et al., 2005; Akkus et al., 2003; Carden et al., 2003; Morris & Mandair 2011; Tarnowski et al., 2004), age-related modifications (Ager et al., 2005; Akkus et al., 2003; Miller et al., 2007), and pathologic or treatment-related changes (Boskey et al., 2005; Carden & Morris 2000; Fratzl 2004; Huang et al., 2003; Siris et al., 2004). The application of Fourier Transform InfraRed Microspectroscopy and Imaging (FTIRM, FTIRI) for bone allows in situ analysis of embedded bone samples at the BSU level. These techniques are based on the vibrations of the atoms of a molecule and give complementary informations. The functional groups present in the sample absorb infrared light (or scatter light for Raman) at different wavelengths. In bone, infrared and Raman spectroscopies were used to obtain information on bone mineral and organic matrix, the two major components of bone. The main advantage of these techniques is that they can be used on embedded bone biopsies, on thin sections (2 μ m-thick MMA sections), keeping the integrity of the bone microarchitecture. By infrared spectroscopy, different vibrations can be analyzed in bone mineral: the phosphate mode vibrations with the $\nu_1\nu_3\text{PO}_4$ corresponding to an antisymmetric stretch, the $\nu_4\text{PO}_4$ (bending stretch), the carbonate vibrations $\nu_2\text{CO}_3$ and $\nu_3\text{CO}_3$. Organic matrix can be analyzed through the Amides I, II and III vibrations. Different parameters can be deduced from these vibrations, as the mineral maturity, crystallinity, carbonate substitution, mineral to matrix ratio, providing informations on bone mineral quality (Boskey et al., 1998, 2005; Miller et al., 2007; Paschalis et al., 1996, 1997a; 1997b; Paschalis & Mendelsohn 2000, Pleshko et al., 1991, Rey et al., 1989, 1995a). By Raman spectroscopy, crystallinity, carbonate type B substitution, and mineral to matrix ratio can be determined (Akkus et al., 2003; Morris & Mandair 2011). Others techniques as vibrational methods have been extensively used to obtain informations on the structural and compound identification, especially on the relative crystallinity (also called crystallinity index).

4.6 Maturity and crystallinity of bone crystals

As mentioned previously, with maturation, stable apatitic domains grow, whereas hydrated layer decreases. Thus, when bone crystals mature, their crystallinity also increases. These two variables can be assessed separately in infrared spectroscopy (Fig.11). Mineral maturity and mineral crystallinity are two parameters temporally linked and often well correlated in

synthetic apatite. The ratio $1030/1020\text{ cm}^{-1}$ (apatitic phosphate over non apatitic phosphate, $\nu_3\text{PO}_4$ vibration) was well correlated in synthetic apatites to the crystallinity measured by XRD, and it was established that the ratio $1030/1020\text{ cm}^{-1}$ was an index of mineral maturity/crystallinity. From that, mineral maturity and crystallinity were associated in a lot of studies (Boskey et al., 2005; Paschalis et al., 1996, 1997a, 1997b). We have defined a new ratio to assess mineral maturity, the $1030/1110\text{ cm}^{-1}$, (apatitic phosphate over non apatitic phosphate, $\nu_3\text{PO}_4$ vibration) equivalent to the $1030/1020\text{ cm}^{-1}$ but more sensitive (Farlay et al., 2010b). We agree with the fact that the $1020/1030\text{ cm}^{-1}$ ratio, which is an index of mineral maturity, evolves simultaneously with crystallinity in synthetic samples or normal bone. However, by definition, those two parameters are different, as mineral maturity corresponds to a stage of maturation, and crystallinity corresponds to the organization of the apatite lattice. We have defined a new mineral crystallinity index, measured by FTIRM on the peak 604 cm^{-1} (bending vibration of phosphates) (Farlay et al., 2010b). This vibration is often inaccessible for microscopic infrared imaging, due to the cut-off of the detector. A wide band detector allows the access to this vibration and we have shown that the value of the full width at half maximum of the 604 cm^{-1} peak, was inversely correlated to the crystallinity (Farlay et al., 2010b). This crystallinity index is well correlated, on the same samples, with another crystallinity index measured on the same vibration, the Shemesh ratio (Shemesh 1990) which is itself derived from the splitting factor of Termine & Posner, 1966. In human bone, it has been shown that those two parameters could evolve separately, and thus can independently affect the mineral characteristics. Indeed, mineral maturity can be affected by modification of bone remodeling (and by formative or antiresorptive treatments), whereas crystallinity can be influenced by ionic substitutions. This was verified in bone samples from patients with skeletal fluorosis (Farlay et al., 2010b). Skeletal fluorosis is a pathology caused by an excessive consumption of fluoride, and characterized by ionic substitution of hydroxyl ions by fluoride ions in bone mineral. In these samples, mineral maturity was decreased, due to a stimulation of osteoblasts on bone formation by fluoride, but crystallinity was increased due to the substitution of OH^- by F^- .

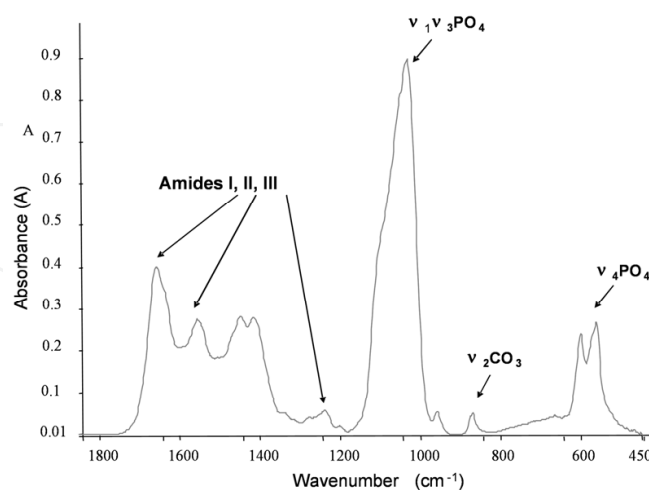


Fig. 11. Infrared spectra of human cortical bone (iliac bone) showing the mineral ($\nu_1\nu_3\text{PO}_4$, $\nu_4\text{PO}_4$, $\nu_2\text{CO}_3$) and the organic (amides) vibrations (INSERM UMR 1033).

In the study previously mentioned for determining the chronology of secondary mineralization on ewes (Bala et al., 2010), it has been shown that kinetic of mineral maturity

was very close to the kinetic of the degree of mineralization, whereas the crystallinity index was biphasic, showing a rapid increase for the six first months, then stabilizing until 18 months, and showing another increase toward highest values after 24 months (Fig.12). In another study performed in women long term-treated by bisphosphonates, an increase in mineral maturity was observed, associated with a decrease in crystallinity, reinforcing the statement that mineral maturity and crystallinity have to be cautiously separately analyzed (Bala et al., 2011).

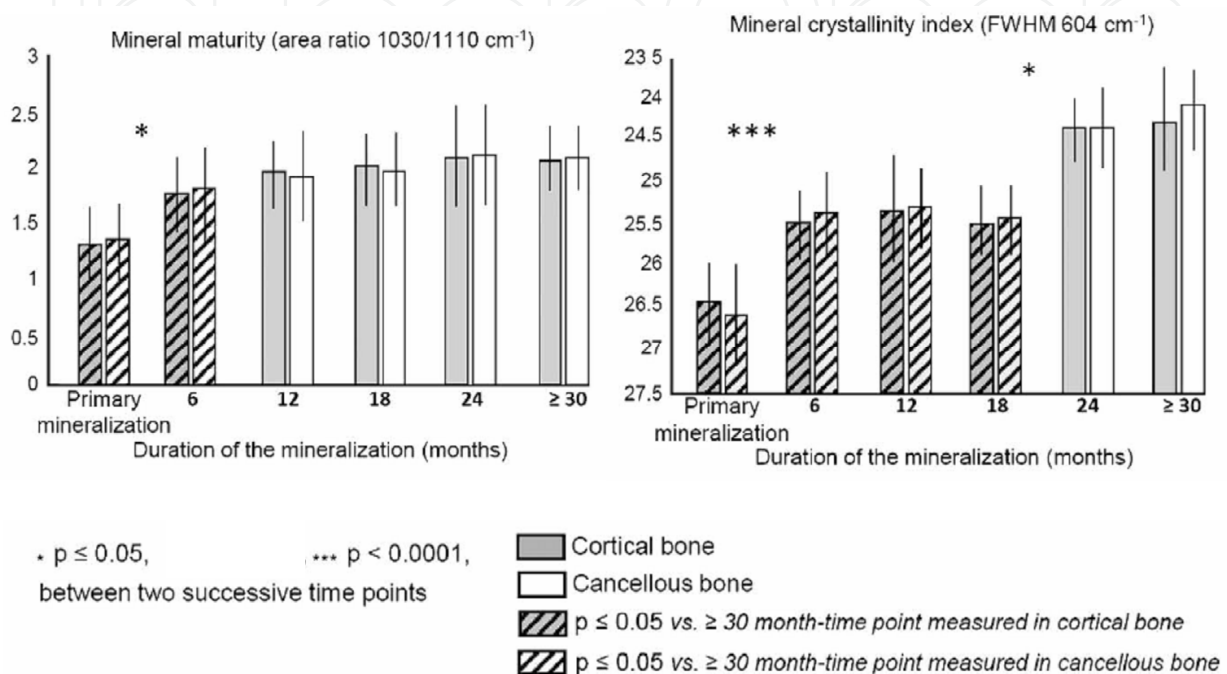


Fig. 12. Histograms showing (left) the rapid increase of mineral maturity during the 6 first months of mineralization, followed by a slowdown and stabilization; (right) the biphasic evolution of mineral crystallinity index, with an increase the 6 first months, a stabilization, and then a resumption at 18 months (Reprinted from Bala et al., 2010, with permission from Elsevier).

Finally, in a study on 53 human vertebrae, it was shown that with age of donor, crystallinity was increased and mineral maturity unchanged (Farlay et al., 2010a). This increase in crystallinity was previously shown in osteoporotic bone (Boskey, 2003), suggesting that with aging, an increase in crystal size/perfection occurs, independently of the bone remodeling (Farlay et al., 2010a).

4.7 Carbonates in bone mineral

In bone mineral, carbonates ions represent about 6-7% of the total mineral ions, thus a non negligible proportion. CO_3^{2-} can be incorporated into bone mineral by substitution in the apatite lattice either to PO_4^{3-} (major site, type-B carbonate) or to OH^- (minor site, type-A carbonate). A third site of CO_3^{2-} ions corresponds to labile carbonates which decrease with the maturation of the apatite crystal (Rey et al., 1989, 1991). The carbonates decrease the regularity of the atomic arrangement in hydroxyapatite (Blumenthal et al., 1975), thus altering the crystallinity. CO_3^{2-} determine physical properties of materials, or biological behavior of cells in scaffold or ceramics. The CO_3^{2-} A/B ratio is very constant among

different species, suggesting that CO_3^{2-} incorporation in bone is a highly regulated process. Carbonates are also important for mineral dissolution (LeGeros 1981). It is well-established in literature that, in synthetic apatites, CO_3^{2-} content increases with time of maturation (Cazalbou et al., 2004b; LeGeros & LeGeros 1983; LeGeros et al., 1968; Rey et al., 1989, 1995a). However, in bone, there is a controversy with some studies showing an increase of CO_3/PO_4 with mineral maturation (Petra et al., 2005) or a decrease (Farlay et al., 2006; Ou-Yang et al., 2001; Paschalis et al., 1996). In very young bone, labile carbonates and HPO_4 are high, and progressively decreased with time of maturation, this being associated with an increase in mineral crystallinity (Magne et al., 2001). The amount of carbonate ions can be measured by different methods (chemical dosage, thermogravimetric analysis, vibrational spectroscopies...). In infrared spectroscopy, carbonate content is generally determined using the $\nu_2\text{CO}_3$ line (out-of-plane bending vibration) which is free of contribution of amide vibrations, and was generally calculated as CO_3/PO_4 area ratio. The role of carbonate in bone mineral is not entirely elucidated. The hypothesis of Wopenka and Pasteris is that carbonate could control bone crystal size, and that high concentration of carbonate could play an important role in constraining bone crystals to the nanometer scale (Wopenka & Pasteris 2005). Indeed, dentin, in which the amount of carbonate concentration is the same than in bone, has also a crystal size similar to bone. However, enamel crystals, which are very much larger size, have only about half the carbonate concentration as bone does.

5. Modifications of bone mineral characteristics with age or in post-menopausal osteoporosis

Bone loss occurs in all individuals after middle life. This bone loss can be moderated (osteopenia), or can become more important (osteoporosis). Osteoporosis is a metabolic bone disease, leading to a decrease in the amount of mineralized bone and an increase in the risk of fracture. In post menopausal osteoporosis, the oestrogen deficiency lead to an acceleration of bone remodeling, the balance between resorption and formation is disturbed in favour of resorption. This lead to a decrease in the more mineralized bone, which is only partially replaced by young less mineralized bone, reducing material stiffness.

5.1 Modifications of bone mineral at tissue level

Several studies performed in bone from osteoporotic women have shown not only that bone mass is decreased but that the bone mineral content was decreased, due to the fact the newly formed BSUs have not the time to achieve their mineralization before to be resorbed. Thus the mean age of matrix is decreased, leading to a decrease to the mean degree of mineralization.

5.1.1 Degree of mineralization (DMB) in control humans (Boivin et al., 2008)

Bone samples from persons of both sexes (sudden died and without known pathology) were studied. This control group was composed of iliac bone samples taken at necropsy from 30 women (aged 48.4 ± 3.7 years; range 20-93 years) and 13 men (aged 66.0 ± 4.4 years; range 43-86 years). The mean DMB expressed in g mineral/ cm^3 (mean \pm SEM) was 1.082 ± 0.017 in cortical bone and 1.099 ± 0.018 in cancellous bone (Figure 13).

In iliac crests, no significant influence of sex or age on the mean DMB was observed (Boivin et al., 2008). In cancellous bone, another study by qBEI showed the absence of influence of

sex, age, ethnicity or skeletal sites in BMDD measurements ($\approx 22\%$ in w% Ca) (Roschger et al., 2003). A recent study on vertebral bone of 53 donors (age range: 54-95 year-old, 21 men, 27 women) showed no influence of sex and age on the DMB (Follet et al., 2010).

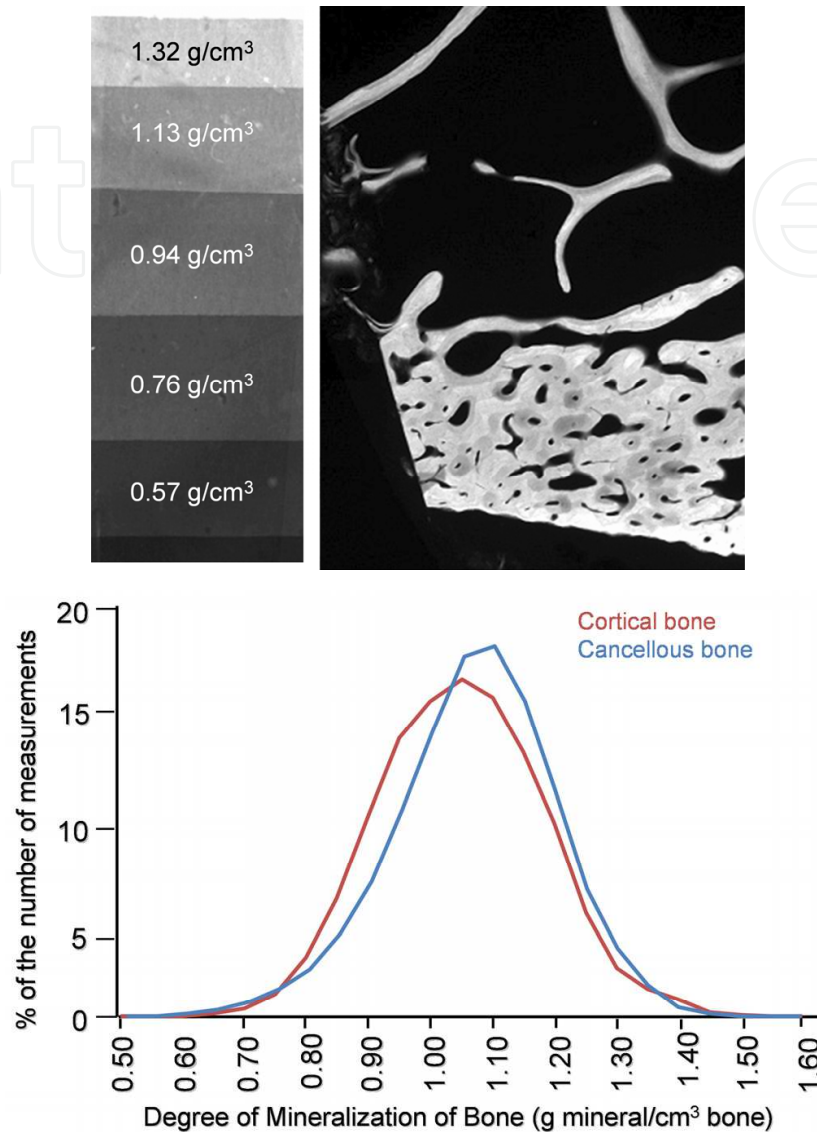


Fig. 13. Quantitative microradiography: (top) aluminium step wedge and microradiograph of bone tissue. (bottom) Distribution of the degrees of mineralization in human iliac bone (INSERM UMR 1033).

5.1.2 Bone mineralization at tissue level in bone of post-menopausal women treated with anti-osteoporotic treatments

In adult bone, the DMB, i.e., the bone mineral density at the tissue level depends on the rate of remodeling. Thus, agents (parathyroid hormone) or events (menopause, ovariectomy) which provoke an augmentation in the « birthrate » or activation frequency of Basic Multicellular Units (BMUs), induce a decrease of the « lifespan » of BSUs, in other words on the time available for the secondary mineralization. This leads to the fact that new BSUs are resorbed before they have fully completed their secondary mineralization, as proven by the presence of a large amount of uncompletely mineralized BSUs and a low mean DMB (Arlot

et al., 2005; Boivin & Meunier 2003; Misof et al., 2003). In a study on men and women with idiopathic osteoporosis, a decrease in DMB was observed compared to controls (-7%) (Boivin et al., 2008). However, a decrease in heterogeneity index of mineralization was observed in this study, indicating a homogeneization of the mineralization. This decrease is also accompanied, in men only, by a decrease in microhardness (-10%) (Boivin et al., 2008). However, it is important to mention that, both in control or osteoporotic bone, no variation in DMB or microhardness was correlated with age. Conversely, antiresorptive agents (bisphosphonates, calcitonin, estrogen, SERMs) which cause a marked reduction in the « birthrate » of BMUs, prolong the « lifespan » of the BSUs, allowing a more complete secondary mineralization and an increase of DMB (Bala et al., 2011a, 2011b; Boivin et al., 2000, 2003; Borah et al., 2005, 2006). A dissociating agent (Strontium Ranelate), acting both on resorption and formation, is now used in the treatment of post-menopausal osteoporosis (Meunier et al., 2004; Reginster et al., 2005). After short- and long-term treatment, the DMB was not significantly different from the physiological range (Boivin et al., 2010; Doublier et al., 2011a). Recently, also with a microradiographic method, it has been shown, in hip-fracture patients, an increase in DMB heterogeneity in both interstitial and osteons compared to controls (Bousson et al., 2011). Thus, some differences exist, considering osteoporotic or hip-fractures cases, in term of heterogeneity of mineralization.

The mineralization index measured by FTIRM, calculated as the ratio of $\nu_1\nu_3\text{PO}_4$ /amide I vibration (Paschalis et al., 1996) is a relative index which has been correlated to ash content (Faibish et al., 2005) and DMB (Farlay et al., unpublished data). The mineralization index (or mineral to matrix ratio) has been shown to be higher in bone of post menopausal woman treated with alendronate (Boskey et al., 2009).

5.2 Modifications of bone crystal characteristics

Concerning crystal characteristics, it has been shown, by XRD technique that, with age, there was an increase in crystal size. In 117 iliac bone samples (range of age: 0-90 years), there was an increase, between 0-30 years, in crystallinity in c-axis, corresponding to the crystal length, and a decrease in the a axis (Hanschin & Stern 1995). After 30 years, modifications were less evident, only a slight increase in crystallinity within the basal plane was observed.

Modifications of bone crystal size impaired mechanical characteristic of bone. Indeed, it was observed that the bones of older animals or that osteoporotic bone, two cases in which bone fractures easily, contained more large crystals than normal (Boskey 2003). Conversely, young animals, which have mechanically strong bones, have a mixture of small and large crystals. In a recent study performed by Raman microspectroscopy in human femurs (age range: 52-85 years old), an overall reduction in heterogeneity of mineralization, crystallinity, and type-B carbonation was observed (Yerramshetty et al., 2006). In this study, bone became more mineralized and more highly type-B carbonated with age, whereas crystallinity was unchanged. In a study performed by FTIRM on 53 human vertebrae (range of age: 54-93 years), a significant increase in crystallinity was observed (Farlay et al., 2010a). This means that, in vertebrae, either crystal size or crystal perfection is increased. In those vertebrae, the bone volume was also significantly decreased. The reason of the increase in crystallinity is unclear, but it is possible that it corresponds to a mechanism of adaptation of bone, in order to compensate the bone loss due to age. In the same study, a decrease in CO_3/PO_4 ratio was observed suggesting an alteration of the physico-chemical composition of bone mineral with age. Thus, comparing the results obtained in human femora and vertebrae in term of

physico-chemical composition, some differences appears. However, the techniques were different (FTIR and Raman microspectroscopies), and the bones were not extracted from the same donors.

6. Perspectives and conclusion

Different factors increase the risk of fracture, independent of bone mineral density. Among these factors, bone mineral features are important in determining some mechanical behavior, especially elastic properties. Besides the decrease in bone mineral volume occurring with age, the alteration of mineral quality plays also a major role in bone fragility. The study of bone mineral quality is an instrumental challenge, due to [1] the dimension and the nature itself of crystal apatite (nanocrystalline), [2] the fact that bone is a composite material (organic matrix intimately linked to bone mineral), and [3] because bone is a complex tissue with different levels of organization (organ, BSU, crystal).

Thus, the using of different complementary techniques (spectroscopic, diffraction, electron microscopy techniques) has been very useful in the comprehension of bone mineral structure the last 50 years. Despite very important findings, there are still lacks in the understanding of the role of certain ions (minor or trace elements) in the biological or biomechanical properties (such as carbonates, strontium ions etc.).

Bone is a fascinating tissue governed by the remodeling activity and the plasma composition. It underlies several characteristics which are interdependent. For example, carbonate ions substitutions into apatite promote the platelet-shape in bone crystals rather than needle-shape found in enamel. But the presence of carbonates, moreover, as mentioned previously, inhibits the incorporation of OH⁻ in bone apatite. Thus, the study of correlations between different variables is still indispensable to understand the relationship between physicochemical and mechanical properties.

From the previous studies performed on bone mineral, it seems that a small crystal size associated with a heterogeneity of crystal size (nanometer size-scale) is important to an optimal mechanical strength. Indeed, the presence of too large crystal is not good for bone strength. Fluoride, as mentioned previously, induces the formation of large crystals, and the formation of a "brittle" bone, despite a high BMD. Moreover, concerning mineralization and its heterogeneity at the tissue level, values comprised between 0.8 and 1.30 g/cm³ of bone could be a great deal to maintain stiffness of the bone. The preservation of mineralization heterogeneity is necessary for bone mechanical strength, allowing, for example, the stopping of microcracks propagation. No modification in degree of mineralization is observed with age or sex, but some differences exist in hip- fracture or osteoporotic cases. Thus a modification in the calcium absorption or an increase in the bone remodeling can lead to a decrease to the degree of mineralization. Differences also exist in studies in degree of mineralization due to the fact that either weight or non-weight-bearing bones are analyzed. We have an ongoing study on these different types of bone (weight or non-weight-bearing bones) in a same donor, in order to analyze the structural and mechanical bone mineral properties.

In conclusion, despite a lot of studies have permitted to understand the characteristics of bone mineral, further studies are needed to clarify the mechanisms of bone fragility at the different levels of investigation.

7. Acknowledgments

The authors gratefully acknowledge Christian Rey, Yohann Bala, Gérard Panczer, Baptiste Depalle, Audrey Doublier, Hélène Follet, Pascale Chavassieux and Pierre Jean Meunier for their collaboration to the major studies referenced in the present chapter.

8. References

- Ager, J. W.; Nalla, R. K.; Breeden, K. L. & Ritchie, R. O. (2005). Deep-ultraviolet Raman spectroscopy study of the effect of aging on human cortical bone. *J Biomed Opt*, Vol.10, No.3, pp. 034012.
- Akkus, O.; Polyakova-Akkus, A.; Adar, F. & Schaffler, M. B. (2003). Aging of microstructural compartments in human compact bone. *J Bone Miner Res*, Vol.18, No.6, pp. 1012-1019.
- Ammann, P. & Rizzoli, R. (2003). Bone strength and its determinants. *Osteoporos Int*, Vol.14 Suppl 3, pp. S13-18.
- Amprino, R. (1958). Investigations on some physical properties of bone tissue. *Acta Anat (Basel)*, Vol.34, No.3, pp. 161-186.
- Arlot, M. ; Meunier, P. J. ; Boivin, G.; Haddock, L.; Tamayo, J.; Correa-Rotter, R.; Jasqui, S.; Donley, D. W.; Dalsky, G. P.; Martin, J. S. & Eriksen, E. F. (2005). Differential effects of teriparatide and alendronate on bone remodeling in postmenopausal women assessed by histomorphometric parameters. *J Bone Miner Res*, Vol.20, No.7, pp. 1244-1253.
- Bala, Y.; Depalle, B.; Douillard, T.; Meille, S.; Clément, P.; Follet, H.; Chevalier, J. & Boivin, G. (2011b). Respective roles of organic and mineral components of human cortical bone matrix in micromechanical behavior: An instrumented indentation study. *J Mech behav biomed materials*, Vol.4, pp. 1473-1482.
- Bala, Y.; Farlay, D.; Chapurlat, R. & Boivin, G. (2011a). Modifications of bone material properties in postmenopausal osteoporotic women long-term treated with alendronate. *Eur J Endocrinol*, Vol 165, No. 4, pp. 647-655
- Bala, Y.; Farlay, D.; Delmas, P. D.; Meunier, P. J. & Boivin, G. (2010). Time sequence of secondary mineralization and microhardness in cortical and cancellous bone from ewes. *Bone*, Vol.46, No.4, pp. 1204-1212. Copyright (2011), with permission from Elsevier.
- Balena, R.; Kleerekoper, M.; Foldes, J. A.; Shih, M. S.; Rao, D. S.; Schober, H. C. & Parfitt, A. M. (1998). Effects of different regimens of sodium fluoride treatment for osteoporosis on the structure, remodeling and mineralization of bone. *Osteoporos Int*, Vol.8, No.5, pp. 428-435.
- Barry, A. B.; Baig, A. A.; Miller, S. C. & Higuchi, W. I. (2002). Effect of age on rat bone solubility and crystallinity. *Calcif Tissue Int*, Vol.71, No.2, pp. 167-171.
- Bertinetti, L.; Drouet, C.; Combes, C.; Rey, C.; Tampieri, A.; Coluccia, S. & Martra, G. (2009). Surface characteristics of nanocrystalline apatites: effect of mg surface enrichment on morphology, surface hydration species & cationic environments. *Langmuir*, Vol.25, No.10, pp. 5647-5654.
- Bigi, A.; Foresti, E.; Gregorini, R.; Ripamonti, A.; Roveri, N. & Shah, J. S. (1992). The role of magnesium on the structure of biological apatites. *Calcif Tissue Int*, Vol.50, No.5, pp. 439-444.
- Blumenthal, N. (1990). The in vitro uptake of trace elements by hydroxyapatite. In: Priest ND, Van de Vyver FL (eds) *Trace Metals and Fluoride in Bones and Teeth*. Vol.CRC Press, Boca Raton, pp. 307-313.

- Blumenthal, N. C.; Betts, F. & Posner, A. S. (1975). Effect of carbonate and biological macromolecules on formation and properties of hydroxyapatite. *Calcif Tissue Res*, Vol.18, No.2, pp. 81-90.
- Blumenthal, N. C.; Betts, F. & Posner, A. S. (1977). Stabilization of amorphous calcium phosphate by Mg and ATP. *Calcif Tissue Res*, Vol.23, No.3, pp. 245-250.
- Blumenthal, N. C. & Posner, A. S. (1984). In vitro model of aluminum-induced osteomalacia: inhibition of hydroxyapatite formation and growth. *Calcif Tissue Int*, Vol.36, No.4, pp. 439-441.
- Boivin, G.; Bala, Y.; Doublier, A.; Farlay, D.; Ste-Marie, L. G.; Meunier, P. J. & Delmas, P. D. (2008). The role of mineralization and organic matrix in the microhardness of bone tissue from controls and osteoporotic patients. *Bone*, Vol.43, No.3, pp. 532-538.
- Boivin, G.; Farlay, D.; Khebbab, M. T.; Jaurand, X.; Delmas, P. D. & Meunier, P. J. (2010). In osteoporotic women treated with strontium ranelate, strontium is located in bone formed during treatment with a maintained degree of mineralization. *Osteoporos Int*, Vol.21, No.4, pp. 667-677.
- Boivin, G.; Lips, P.; Ott, S. M.; Harper, K. D.; Sarkar, S.; Pinette, K. V. & Meunier, P. J. (2003). Contribution of raloxifene and calcium and vitamin D3 supplementation to the increase of the degree of mineralization of bone in postmenopausal women. *J Clin Endocrinol Metab*, Vol.88, No.9, pp. 4199-4205.
- Boivin, G. & Meunier, P. J. (2002). The degree of mineralization of bone tissue measured by computerized quantitative contact microradiography. *Calcif Tissue Int*, Vol.70, No.6, pp. 503-511.
- Boivin, G. & Meunier, P. J. (2003). Methodological considerations in measurement of bone mineral content. *Osteoporos Int*, Vol.14 Suppl 5, pp. S22-7; discussion S27-28.
- Boivin, G. Y.; Chavassieux, P. M.; Santora, A. C.; Yates, J. & Meunier, P. J. (2000). Alendronate increases bone strength by increasing the mean degree of mineralization of bone tissue in osteoporotic women. *Bone*, Vol.27, No.5, pp. 687-694.
- Borah, B.; Dufresne, T. E.; Ritman, E. L.; Jorgensen, S. M.; Liu, S.; Chmielewski, P. A.; Phipps, R. J.; Zhou, X.; Sibonga, J. D. & Turner, R. T. (2006). Long-term risedronate treatment normalizes mineralization and continues to preserve trabecular architecture: sequential triple biopsy studies with micro-computed tomography. *Bone*, Vol.39, No.2, pp. 345-352.
- Borah, B.; Ritman, E. L.; Dufresne, T. E.; Jorgensen, S. M.; Liu, S.; Sacha, J.; Phipps, R. J. & Turner, R. T. (2005). The effect of risedronate on bone mineralization as measured by micro-computed tomography with synchrotron radiation: correlation to histomorphometric indices of turnover. *Bone*, Vol.37, No.1, pp. 1-9.
- Boskey, A. (2003). Bone mineral crystal size. *Osteoporos Int*, Vol.14 Suppl 5, pp. S16-20; discussion S20-1.
- Boskey, A.; Maresca, M. & Appel, J. (1989). The effects of noncollagenous matrix proteins on hydroxyapatite formation and proliferation in a collagen gel system. *Connect Tissue Res*, Vol.21, No.1-4, pp. 171-176; discussion 177-8.
- Boskey, A. L. (1989). Noncollagenous matrix proteins and their role in mineralization. *Bone Miner*, Vol.6, No.2, pp. 111-123.
- Boskey, A. L.; DiCarlo, E.; Paschalis, E.; West, P. & Mendelsohn, R. (2005). Comparison of mineral quality and quantity in iliac crest biopsies from high- and low-turnover osteoporosis: an FT-IR microspectroscopic investigation. *Osteoporos Int*, Vol.16, No.12, pp. 2031-2038.

- Boskey, A. L.; Gadaleta, S.; Gundberg, C.; Doty, S. B.; Ducey, P. & Karsenty, G. (1998). Fourier transform infrared microspectroscopic analysis of bones of osteocalcin-deficient mice provides insight into the function of osteocalcin. *Bone*, Vol.23, No.3, pp. 187-196.
- Boskey, A. L.; Rimnac, C. M.; Bansal, M.; Federman, M.; Lian, J. & Boyan, B. D. (1992). Effect of short-term hypomagnesemia on the chemical and mechanical properties of rat bone. *J Orthop Res*, Vol.10, No.6, pp. 774-783.
- Boskey, A. L.; Spevak, L. & Weinstein, R. S. (2009). Spectroscopic markers of bone quality in alendronate-treated postmenopausal women. *Osteoporos Int*, Vol.20, No.5, pp. 793-800.
- Bousson, V.; Bergot, C.; Wu, Y.; Jolivet, E.; Zhou, L. Q. & Laredo, J. D. (2011). Greater tissue mineralization heterogeneity in femoral neck cortex from hip-fractured females than controls. A microradiographic study. *Bone*, Vol.48, No.6, pp. 1252-1259.
- Bouxsein, M. L. (2005). Determinants of skeletal fragility. *Best Pract Res Clin Rheumatol*, Vol.19, No.6, pp. 897-911.
- Carden, A. & Morris, M. D. (2000). Application of vibrational spectroscopy to the study of mineralized tissues (review). *J Biomed Opt*, Vol.5, No.3, pp. 259-268.
- Carden, A.; Rajachar, R. M.; Morris, M. D. & Kohn, D. H. (2003). Ultrastructural changes accompanying the mechanical deformation of bone tissue: a Raman imaging study. *Calcif Tissue Int*, Vol.72, No.2, pp. 166-175.
- Carlstrom, D. (1954). Micro-hardness measurements on single haversian systems in bone. *Experientia*, Vol.10, No.4, pp. 171-172.
- Cazalbou, S.; Combes, C.; Eichert, D. & Rey, C. (2004a). Adaptive physico-chemistry of bio(related calcium phosphates. *J Mater Chem*, Vol.14, pp. 2148-2153.
- Cazalbou, S.; Combes, C.; Eichert, D.; Rey, C. & Glimcher, M. J. (2004b). Poorly crystalline apatites: evolution and maturation in vitro and in vivo. *J Bone Miner Metab*, Vol.22, No.4, pp. 310-317.
- Cho, G.; Wu, Y. & Ackerman, J. L. (2003). Detection of hydroxyl ions in bone mineral by solid-state NMR spectroscopy. *Science*, Vol.300, No.5622, pp. 1123-1127.
- Currey, J. D. (2003). How well are bones designed to resist fracture? *J Bone Miner Res*, Vol.18, No.4, pp. 591-598.
- Doublier, A.; Farlay, D.; Jaurand, X.; Vera, R. & Boivin, G. (2011b). Effects of strontium on the quality of bone apatite crystals. *33rd Annual meeting of American Society for Bone and Mineral Research, San Diego, California, USA, September 16-20, 2011.*
- Doublier, A.; Farlay, D.; Khebbab, M. T.; Jaurand, X.; Meunier, P. J. & Boivin, G. (2011a). Distribution of Strontium and Mineralization in Iliac Bone Biopsies from Osteoporotic Women Long-Term Treated with Strontium Ranelate. *Eur J Endocrinol*, Vol 165, No 3, pp. 469-476.
- Elliott, S. R. & Robinson, R. A. (1957). The water content of bone. I. The mass of water, inorganic crystals, organic matrix, and CO₂ space components in a unit volume of the dog bone. *J Bone Joint Surg Am*, Vol.39-A, No.1, pp. 167-88.
- Faibish, D.; Gomes, A.; Boivin, G.; Binderman, I. & Boskey, A. (2005). Infrared imaging of calcified tissue in bone biopsies from adults with osteomalacia. *Bone*, Vol.36, No.1, pp. 6-12.
- Farlay, D.; Bala, Y.; Burt-Pichat, B.; Chapurlat, R.; Boivin, G. & Follet, H. (2010a). Relationship Between Age, Mineral Characteristics, Mineralization, Microhardness, And Microcracks In Human Vertebral Trabecular Bone. *32nd annual meeting of American Society for Bone and Mineral Research. Toronto, Canada, October 15-19, 2010.*

- Farlay, D.; Panczer, G.; Rey, C.; Delmas, P. D. & Boivin, G. (2006). New characteristics of bone mineral validated by infrared microspectroscopy. *28th annual meeting of American Society for Bone and Mineral Research*, Philadelphia, Pennsylvania, USA, September 19-22, 2006.
- Farlay, D.; Panczer, G.; Rey, C.; Delmas, P. D. & Boivin, G. (2010b). Mineral maturity and crystallinity index are distinct characteristics of bone mineral. *J Bone Miner Metab*, Vol.28, No.4, pp. 433-445.
- Follet, H.; Boivin, G.; Rumelhart, C. & Meunier, P. J. (2004). The degree of mineralization is a determinant of bone strength: a study on human calcanei. *Bone*, Vol.34, No.5, pp. 783-789.
- Follet, H.; Viguet-Carrin, S.; Burt-Pichat, B.; Depalle, B.; Bala, Y.; Gineyts, E.; Munoz, F.; Arlot, M.; Boivin, G.; Chapurlat, R. D.; Delmas, P. D. & Bouxsein, M. L. (2010). Effects of preexisting microdamage, collagen cross-links, degree of mineralization, age, and architecture on compressive mechanical properties of elderly human vertebral trabecular bone. *J Orthop Res*, Vol.29, No.4, pp. 481-488.
- Fratzl, P.; Fratzl-Zelman, N.; Klaushofer, K. (1993) Collagen packing and mineralization. An x-ray scattering investigation of turkey leg tendon. *Biophys J*, Vol.64, No 1, pp 260-266.
- Fratzl, P.; Gupta, HS, Paschalis EP, Roschger P. (2004). Structure and mechanical quality of the mineral quality of the collagen-mineral nano-composite in bone. *J Mater Chem*, Vol.14, pp. 2115-2123.
- Frost, H. M. (1969). Tetracycline-based histological analysis of bone remodeling. *Calcif Tissue Res*, Vol.3, No.3, pp. 211-237.
- Fuchs, R. K.; Allen, M. R.; Ruppel, M. E.; Diab, T.; Phipps, R. J.; Miller, L. M. & Burr, D. B. (2008). In situ examination of the time-course for secondary mineralization of Haversian bone using synchrotron Fourier transform infrared microspectroscopy. *Matrix Biol*, Vol.27, No.1, pp. 34-41.
- George, A. & Veis, A. (2008). Phosphorylated proteins and control over apatite nucleation, crystal growth, and inhibition. *Chem Rev*, Vol.108, No.11, pp. 4670-4693.
- Glimcher, M. G. (1998). The nature of the mineral phase in bone: Biological and clinical implications. *Metabolic bone disease*, Vol.Chap. 2, pp. 23-50.
- Glimcher, M. J. (2006). Bone: Nature of the calcium phosphate crystals and cellular, structural, and physical chemical mechanisms in their formation. *Rev Mineral Geochem*, Vol.64, pp. 223-282.
- Glimcher, M. J.; Hodge, A. J. & Schmitt, F. O. (1957). Macromolecular Aggregation States in Relation to Mineralization: the Collagen-Hydroxyapatite System as Studied in Vitro. *Proc Natl Acad Sci U S A*, Vol.43, No.10, pp. 860-867.
- Glorieux, F. H.; Travers, R.; Taylor, A.; Bowen, J. R.; Rauch, F.; Norman, M. & Parfitt, A. M. (2000). Normative data for iliac bone histomorphometry in growing children. *Bone*, Vol.26, No.2, pp. 103-109.
- Grynepas, M. D. & Marie, P. J. (1990). Effects of low doses of strontium on bone quality and quantity in rats. *Bone*, Vol.11, No.5, pp. 313-319.
- Guggenbuhl, P.; Filmon, R.; Mabileau, G.; Basle, M. F. & Chappard, D. (2008). Iron inhibits hydroxyapatite crystal growth in vitro. *Metabolism*, Vol.57, No.7, pp. 903-910.
- Hanschin, R. G. & Stern, W. B. (1995). X-ray diffraction studies on the lattice perfection of human bone apatite (Crista iliaca). *Bone*, Vol.16, No.4 Suppl, pp. 355S-363S.
- Höhling, H. L.; Barckhaus, R. H.; Krefting, E. R.; Althoff, J. & Quint, P. (1990). Collagen mineralization: Aspects of the structural relationships between collagen and the

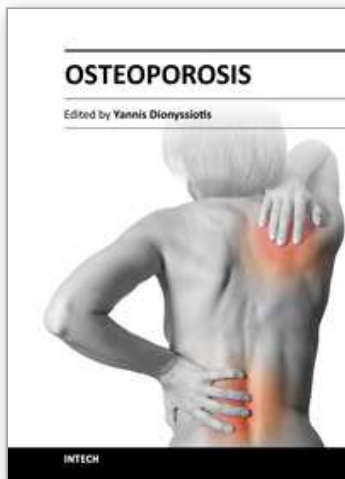
- apatitic crystallites. In "Ultrastructure of skeletal tissues (E. Bonucci and P. M. Motta, Eds). Academic Press; San Diego.; pp. 41-62.
- Huang, R. Y.; Miller, L. M.; Carlson, C. S. & Chance, M. R. (2003). In situ chemistry of osteoporosis revealed by synchrotron infrared microspectroscopy. *Bone*, Vol.33, No.4, pp. 514-521.
- Jager, C.; Welzel, T.; Meyer-Zaika, W. & Epple, M. (2006). A solid-state NMR investigation of the structure of nanocrystalline hydroxyapatite. *Magn Reson Chem*, Vol.44, No.6, pp. 573-580.
- Jonsson, U.; Ranta, H. & Stromberg, L. (1985). Growth changes of collagen cross-linking, calcium, and water content in bone. *Arch Orthop Trauma Surg*, Vol.104, No.2, pp. 89-93.
- Landis, W. J. (1995). The strength of a calcified tissue depends in part on the molecular structure and organization of its constituent mineral crystals in their organic matrix. *Bone*, Vol.16, No.5, pp. 533-544.
- Landis, W. J.; Song, M. J.; Leith, A.; McEwen, L. & McEwen, B. F. (1993). Mineral and organic matrix interaction in normally calcifying tendon visualized in three dimensions by high-voltage electron microscopic tomography and graphic image reconstruction. *J Struct Biol*, Vol.110, No.1, pp. 39-54.
- Le Bail, A. & Loüer, D. (1978). Smoothing and validity of crystallite size distributions from X-ray line profile analysis. *J Appl Crystallogr*, Vol.11, pp. 50-55.
- Lees, S. (1987). Considerations regarding the structure of the mammalian mineralized osteoid from viewpoint of the generalized packing model. *Connect Tissue Res*, Vol.16, No.4, pp. 281-303.
- LeGeros, R. Z. (1981). Apatites in biological systems. *Prog.Crystal Growth Charact.*; Vol.4, pp. 1-45.
- LeGeros, R. Z.; Bonel, G. & Legros, R. (1978). Types of "H₂O" in human enamel and in precipitated apatites. *Calcif Tissue Res*, Vol.26, No.2, pp. 111-118.
- LeGeros, R. Z. & LeGeros, J. P. (1983). Carbonate analysis of synthetic mineral and biological apatites. *J Dent Res*, Vol.62, pp. 259.
- LeGeros, R. Z.; Trautz, O. R.; Legeros, J. P. & Klein, E. (1968). Carbonate substitution in the apatitic structure. *Bull Soc Chim Fr*, Vol.4, pp. 1712-1718.
- Li, C.; Paris, O.; Siegel, S.; Roschger, P.; Paschalis, E. P.; Klaushofer, K. & Fratzl, P. (2010a). Strontium is incorporated into mineral crystals only in newly formed bone during strontium ranelate treatment. *J Bone Miner Res*, Vol.25, No.5, pp. 968-975.
- Li, X.; Hu, C.; Zhu, Y.; Sun, H.; Li, Y. & Zhang, Z. (2010b). Effects of Aluminum Exposure on Bone Mineral Density, Mineral & Trace Elements in Rats. *Biol Trace Elem Res*. doi 10.2007/s12011-010-8861-4.
- Li, Z. Y.; Lam, W. M.; Yang, C.; Xu, B.; Ni, G. X.; Abbah, S. A.; Cheung, K. M.; Luk, K. D. & Lu, W. W. (2007). Chemical composition, crystal size and lattice structural changes after incorporation of strontium into biomimetic apatite. *Biomaterials*, Vol.28, No.7, pp. 1452-1460. Copyright (2011), with permission from Elsevier.
- Loong, C. K.; Rey, C.; Kuhn, L. T.; Combes, C.; Wu, Y.; Chen, S. & Glimcher, M. J. (2000). Evidence of hydroxyl-ion deficiency in bone apatites: an inelastic neutron-scattering study. *Bone*, Vol.26, No.6, pp. 599-602.
- Magne, D.; Pilet, P.; Weiss, P. & Daculsi, G. (2001). Fourier transform infrared microspectroscopic investigation of the maturation of nonstoichiometric apatites in mineralized tissues: a horse dentin study. *Bone*, Vol.29, No.6, pp. 547-552.

- Malaval, L.; Wade-Gueye, N. M.; Boudiffa, M.; Fei, J.; Zirngibl, R.; Chen, F.; Laroche, N.; Roux, J. P.; Burt-Pichat, B.; Duboeuf, F.; Boivin, G.; Jurdic, P.; Lafage-Proust, M. H.; Amedee, J.; Vico, L.; Rossant, J. & Aubin, J. E. (2008). Bone sialoprotein plays a functional role in bone formation and osteoclastogenesis. *J Exp Med*, Vol.205, No.5, pp. 1145-1153.
- Marie, P. J.; Hott, M.; Modrowski, D.; De Pollak, C.; Guillemain, J.; Deloffre, P. & Tsouderos, Y. (1993). An uncoupling agent containing strontium prevents bone loss by depressing bone resorption and maintaining bone formation in estrogen-deficient rats. *J Bone Miner Res*, Vol.8, No.5, pp. 607-615.
- Meunier, P. J. & Boivin, G. (1997). Bone mineral density reflects bone mass but also the degree of mineralization of bone: therapeutic implications. *Bone*, Vol.21, No.5, pp. 373-377.
- Meunier, P. J.; Roux, C.; Seeman, E.; Ortolani, S.; Badurski, J. E.; Spector, T. D.; Cannata, J.; Balogh, A.; Lemmel, E. M.; Pors-Nielsen, S.; Rizzoli, R.; Genant, H. K. & Reginster, J. Y. (2004). The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. *N Engl J Med*, Vol.350, No.5, pp. 459-468.
- Miller, L. M.; Little, W.; Schirmer, A.; Sheik, F.; Busa, B. & Judex, S. (2007). Accretion of bone quantity and quality in the developing mouse skeleton. *J Bone Miner Res*, Vol.22, No.7, pp. 1037-1045.
- Miller, L. M.; Vairavamurthy, V.; Chance, M. R.; Mendelsohn, R.; Paschalis, E. P.; Betts, F. & Boskey, A. L. (2001). In situ analysis of mineral content and crystallinity in bone using infrared micro-spectroscopy of the nu(4) PO(4)(3-) vibration. *Biochim Biophys Acta*, Vol.1527, No.1-2, pp. 11-19.
- Misof, B. M.; Roschger, P.; Cosman, F.; Kurland, E. S.; Tesch, W.; Messmer, P.; Dempster, D. W.; Nieves, J.; Shane, E.; Fratzl, P.; Klaushofer, K.; Bilezikian, J. & Lindsay, R. (2003). Effects of intermittent parathyroid hormone administration on bone mineralization density in iliac crest biopsies from patients with osteoporosis: a paired study before and after treatment. *J Clin Endocrinol Metab*, Vol.88, No.3, pp. 1150-1156.
- Morris, M. D. & Mandair, G. S. (2011). Raman assessment of bone quality. *Clin Orthop Relat Res*, Vol.469, No.8, pp. 2160-2169.
- Mueller, K. H.; Trias, A. & Ray, R. D. (1966). Bone density and composition. Age-related and pathological changes in water and mineral content. *J Bone Joint Surg Am*, Vol.48, No.1, pp. 140-148.
- Myllyharju, J. & Kivirikko, K. I. (2004). Collagens, modifying enzymes and their mutations in humans, flies and worms. *Trends Genet*, Vol.20, No.1, pp. 33-43.
- Naito, A.; Tuzi, S. & Saito, H. (1994). A high-resolution ¹⁵N solid-state NMR study of collagen and related polypeptides. The effect of hydration on formation of interchain hydrogen bonds as the primary source of stability of the collagen-type triple helix. *Eur J Biochem*, Vol.224, No.2, pp. 729-734.
- Nalla, R. K.; Kinney, J. H. & Ritchie, R. O. (2003). Mechanistic fracture criteria for the failure of human cortical bone. *Nat Mater*, Vol.2, No.3, pp. 164-168.
- Nuzzo, S.; Peyrin, F.; Cloetens, P.; Baruchel, J. & Boivin, G. (2002). Quantification of the degree of mineralization of bone in three dimensions using synchrotron radiation microtomography. *Med Phys*, Vol.29, No.11, pp. 2672-2681.
- Nyman, J. S.; Roy, A.; Shen, X.; Acuna, R. L.; Tyler, J. H. & Wang, X. (2006). The influence of water removal on the strength and toughness of cortical bone. *J Biomech*, Vol.39, No.5, pp. 931-938.

- Oliver, W. & Pharr, G. (1992). An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. *J Mater Res*, Vol.7, No.6, pp. 1564-1593.
- Omelon, S.; Georgiou, J.; Henneman, Z. J.; Wise, L. M.; Sukhu, B.; Hunt, T.; Wynnyckyj, C.; Holmyard, D.; Bielecki, R. & Grynpas, M. D. (2009). Control of vertebrate skeletal mineralization by polyphosphates. *PLoS One*, Vol.4, No.5, e5634.
- Ou-Yang, H.; Paschalis, E. P.; Mayo, W. E.; Boskey, A. L. & Mendelsohn, R. (2001). Infrared microscopic imaging of bone: spatial distribution of CO₃(²⁻). *J Bone Miner Res*, Vol.16, No.5, pp. 893-900.
- Oyen, M. L. (2006). Nanoindentation hardness of mineralized tissues. *J Biomech*, Vol.39, No.14, pp. 2699-2702.
- Paschalis, E. P.; Betts, F.; DiCarlo, E.; Mendelsohn, R. & Boskey, A. L. (1997a). FTIR microspectroscopic analysis of human iliac crest biopsies from untreated osteoporotic bone. *Calcif Tissue Int*, Vol.61, No.6, pp. 487-492.
- Paschalis, E. P.; Betts, F.; DiCarlo, E.; Mendelsohn, R. & Boskey, A. L. (1997b). FTIR microspectroscopic analysis of normal human cortical and trabecular bone. *Calcif Tissue Int*, Vol.61, No.6, pp. 480-486.
- Paschalis, E. P.; DiCarlo, E.; Betts, F.; Sherman, P.; Mendelsohn, R. & Boskey, A. L. (1996). FTIR microspectroscopic analysis of human osteonal bone. *Calcif Tissue Int*, Vol.59, No.6, pp. 480-487.
- Paschalis, E. P. & Mendelsohn, R. (2000). Variations in the individual thick lamellar properties within osteons by nanodentation. *Bone*, Vol.26, No.5, pp. 545-546.
- Pasteris, J. D.; Wopenka, B.; Freeman, J. J.; Rogers, K.; Valsami-Jones, E.; van der Houwen, J. A. & Silva, M. J. (2004). Lack of OH in nanocrystalline apatite as a function of degree of atomic order: implications for bone and biomaterials. *Biomaterials*, Vol.25, No.2, pp. 229-238.
- Petra, M.; Anastassopoulou, J.; Theologis, T. & Theophanides, T. (2005). Synchrotron micro-FT-IR spectroscopic evaluation of normal paediatric human bone. *J Molec Struct*, Vol.733, No.101-110.
- Pleshko, N.; Boskey, A. & Mendelsohn, R. (1991). Novel infrared spectroscopic method for the determination of crystallinity of hydroxyapatite minerals. *Biophys J*, Vol.60, No.4, pp. 786-793.
- Posner, A. (1985). The structure of bone apatite surfaces. *J Biomed Materials Res* Vol.19, pp. 241-250.
- Posner, A. S. (1969). Crystal chemistry of bone mineral. *Physiological reviews*, Vol.69, No.4, pp. 760-787.
- Powder diffraction file (PDF), I. C. f. D. D.; 12 campus blvd, Newton square, PA 19073-3273, USA, <http://www.idd.com>.
- Rauch, F. & Glorieux, F. H. (2004). Osteogenesis imperfecta. *Lancet*, Vol.363, No.9418, pp. 1377-1385.
- Reginster, J. Y.; Seeman, E.; De Vernejoul, M. C.; Adami, S.; Compston, J.; Phenekos, C.; Devogelaer, J. P.; Curiel, M. D.; Sawicki, A.; Goemaere, S.; Sorensen, O. H.; Felsenberg, D. & Meunier, P. J. (2005). Strontium ranelate reduces the risk of nonvertebral fractures in postmenopausal women with osteoporosis: Treatment of Peripheral Osteoporosis (TROPOS) study. *J Clin Endocrinol Metab*, Vol.90, No.5, pp. 2816-2822.

- Rey, C.; Collins, B.; Goehl, T.; Dickson, I. R. & Glimcher, M. J. (1989). The carbonate environment in bone mineral: a resolution-enhanced Fourier Transform Infrared Spectroscopy Study. *Calcif Tissue Int*, Vol.45, No.3, pp. 157-164.
- Rey, C.; Combes, C.; Drouet, C. & Glimcher, M. J. (2009). Bone mineral: update on chemical composition and structure. *Osteoporos Int*, Vol.20, No.6, pp. 1013-1021.
- Rey, C.; Hina, A.; Tofighi, A.; Glimcher M.J. (1995a). Maturation of poorly crystalline apatites: chemical and structural aspects in vivo and in vitro. *Cells and Mater.*; Vol.5, pp. 345-356.
- Rey, C.; Miquel, J. L.; Facchini, L.; Legrand, A. P. & Glimcher, M. J. (1995b). Hydroxyl groups in bone mineral. *Bone*, Vol.16, No.5, pp. 583-586.
- Rey, C.; Renugopalakrishnan, V.; Shimizu, M.; Collins, B. & Glimcher, M. J. (1991). A resolution-enhanced Fourier transform infrared spectroscopic study of the environment of the CO₃(²⁻) ion in the mineral phase of enamel during its formation and maturation. *Calcif Tissue Int*, Vol.49, No.4, pp. 259-268.
- Rey, C.; Shimizu, M.; Collins, B. & Glimcher, M. J. (1990). Resolution-enhanced Fourier transform infrared spectroscopy study of the environment of phosphate ions in the early deposits of a solid phase of calcium-phosphate in bone and enamel, and their evolution with age. I: Investigations in the epsilon 4 PO₄ domain. *Calcif Tissue Int*, Vol.46, No.6, pp. 384-394.
- Rietveld, H. (1969). A profile refinement method for nuclear and magnetic structure. *J Appl Crystallogr*, Vol.2, pp. 65-71.
- Riggs, C. M.; Lanyon, L. E. & Boyde, A. (1993). Functional associations between collagen fibre orientation and locomotor strain direction in cortical bone of the equine radius. *Anat Embryol (Berl)*, Vol.187, No.3, pp. 231-238.
- Robinson, R. A. (1975). Physiocochemical structure of bone. *Clin Orthop Relat Res*, No.112, pp. 263-315.
- Roschger, P.; Fratzl, P.; Eschberger, J. & Klaushofer, K. (1998). Validation of quantitative backscattered electron imaging for the measurement of mineral density distribution in human bone biopsies. *Bone*, Vol.23, No.4, pp. 319-326.
- Roschger, P.; Gupta, H. S.; Berzlanovich, A.; Ittner, G.; Dempster, D. W.; Fratzl, P.; Cosman, F.; Parisien, M.; Lindsay, R.; Nieves, J. W. & Klaushofer, K. (2003). Constant mineralization density distribution in cancellous human bone. *Bone*, Vol.32, No.3, pp. 316-323.
- Roschger, P.; Plenk, H.; Jr.; Klaushofer, K. & Eschberger, J. (1995). A new scanning electron microscopy approach to the quantification of bone mineral distribution: backscattered electron image grey-levels correlated to calcium K alpha-line intensities. *Scanning Microsc*, Vol.9, No.1, pp. 75-86; discussion 86-8.
- Ruffoni, D.; Fratzl, P.; Roschger, P.; Klaushofer, K. & Weinkamer, R. (2007). The bone mineralization density distribution as a fingerprint of the mineralization process. *Bone*, Vol.40, No.5, pp. 1308-1319.
- Saito, H.; Yokoi, M. (1992) A ¹³C NMR study on collagens in the solid state: hydration/dehydration-induced conformational change of collagen and detection of internal motions. *J Biochem*, Vol. 111, No 3, pp 376-382.
- Saito, H.; Tabeta, R.; Shoji, A.; Ozaki, T.; Ando, I.; Miyata, T. (1984) A high-resolution ¹³C-NMR study of collagenlike polypeptides and collagen fibrils in solid state studied by the cross-polarization-magic angle-spinning method. Manifestation of conformation-dependent ¹³C chemical shifts and application to conformational characterization. *Biopolymers*, Vol. 23, pp 2279-2297.

- Saito, M. & Marumo, K. (2010). Collagen cross-links as a determinant of bone quality: a possible explanation for bone fragility in aging, osteoporosis, and diabetes mellitus. *Osteoporos Int*, Vol.21, No.2, pp. 195-214.
- Seeman, E. & Delmas, P. D. (2006). Bone quality--the material and structural basis of bone strength and fragility. *N Engl J Med*, Vol.354, No.21, pp. 2250-2261.
- Shemesh, A. (1990). Crystallinity and diagenesis of sedimentary apatites. *Geochimica et Cosmochimica Acta*, Vol.54, No.9, pp. 2433-2438.
- Siris, E. S.; Brennan, S. K.; Miller, P. D.; Barrett-Connor, E.; Chen, Y. T.; Sherwood, L. M. & Abbott, T. A. (2004). Predictive value of low BMD for 1-year fracture outcomes is similar for postmenopausal women ages 50-64 and 65 and Older: results from the National Osteoporosis Risk Assessment (NORA). *J Bone Miner Res*, Vol.19, No.8, pp. 1215-1220.
- Sornay-Rendu, E.; Munoz, F.; Garnero, P.; Duboeuf, F. & Delmas, P. D. (2005). Identification of osteopenic women at high risk of fracture: the OFELY study. *J Bone Miner Res*, Vol.20, No.10, pp. 1813-1819.
- Tarnowski, C. P.; Ignelzi, M. A.; Jr.; Wang, W.; Taboas, J. M.; Goldstein, S. A. & Morris, M. D. (2004). Earliest mineral and matrix changes in force-induced musculoskeletal disease as revealed by Raman microspectroscopic imaging. *J Bone Miner Res*, Vol.19, No.1, pp. 64-71.
- Termine, J. D.; Eanes, E. D.; Greenfield, D. J.; Nylen, M. U. & Harper, R. A. (1973). Hydrazine-deproteinated bone mineral. Physical and chemical properties. *Calcif Tissue Res*, Vol.12, No.1, pp. 73-90.
- Termine, J. D. & Posner, A. S. (1966). Infra-red determination of the percentage of crystallinity in apatitic calcium phosphates. *Nature*, Vol.211, No.5046, pp. 268-270.
- Traub, W.; Arad, T. & Weiner, S. (1992). Origin of mineral crystal growth in collagen fibrils. *Matrix*, Vol.12, No.4, pp. 251-255.
- Turner, C. H. & Burr, D. B. (1993). Basic biomechanical measurements of bone: a tutorial. *Bone*, Vol.14, No.4, pp. 595-608. Copyright (2011), with permission from Elsevier.
- Vedi, S.; Compston, J. E.; Webb, A. & Tighe, J. R. (1983). Histomorphometric analysis of dynamic parameters of trabecular bone formation in the iliac crest of normal British subjects. *Metab Bone Dis Relat Res*, Vol.5, No.2, pp. 69-74.
- Viguet-Carrin, S.; Garnero, P. & Delmas, P. D. (2006). The role of collagen in bone strength. *Osteoporos Int*, Vol.17, No.3, pp. 319-336.
- Weaver, J. K. (1966). The microscopic hardness of bone. *J Bone Joint Surg Am*, Vol.48, No.2, pp. 273-288.
- Wopenka, B. & Pasteris, J. D. (2005). A mineralogical perspective on the apatite in bone. *Mater. Sci. Eng. C*, Vol.25, pp. 131-143.
- Yerramshetty, J. S.; Lind, C. & Akkus, O. (2006). The compositional and physicochemical homogeneity of male femoral cortex increases after the sixth decade. *Bone*, Vol.39, No.6, pp. 1236-1243.
- Zhu, P.; Xu, J.; Sahar, N.; Morris, MD., Kohn, DH., Ramamoorthy, A. (2009) Time-resolved dehydration-induced structural changes in an intact bovine cortical bone revealed by solid-state NMR spectroscopy. *J Am Chem Soc*, Vol.131, No 7, pp 17064-17065.



Osteoporosis

Edited by PhD. Yannis Dionyssiotis

ISBN 978-953-51-0026-3

Hard cover, 864 pages

Publisher InTech

Published online 24, February, 2012

Published in print edition February, 2012

Osteoporosis is a public health issue worldwide. During the last few years, progress has been made concerning the knowledge of the pathophysiological mechanism of the disease. Sophisticated technologies have added important information in bone mineral density measurements and, additionally, geometrical and mechanical properties of bone. New bone indices have been developed from biochemical and hormonal measurements in order to investigate bone metabolism. Although it is clear that drugs are an essential element of the therapy, beyond medication there are other interventions in the management of the disease. Prevention of osteoporosis starts in young ages and continues during aging in order to prevent fractures associated with impaired quality of life, physical decline, mortality, and high cost for the health system. A number of different specialties are holding the scientific knowledge in osteoporosis. For this reason, we have collected papers from scientific departments all over the world for this book. The book includes up-to-date information about basics of bones, epidemiological data, diagnosis and assessment of osteoporosis, secondary osteoporosis, pediatric issues, prevention and treatment strategies, and research papers from osteoporotic fields.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Delphine Farlay and Georges Boivin (2012). Bone Mineral Quality, Osteoporosis, PhD. Yannis Dionyssiotis (Ed.), ISBN: 978-953-51-0026-3, InTech, Available from: <http://www.intechopen.com/books/osteoporosis/bone-mineral-quality>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen