Study of bone structure and composition by analytical techniques

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ANALYTICAL TECHNIQUES

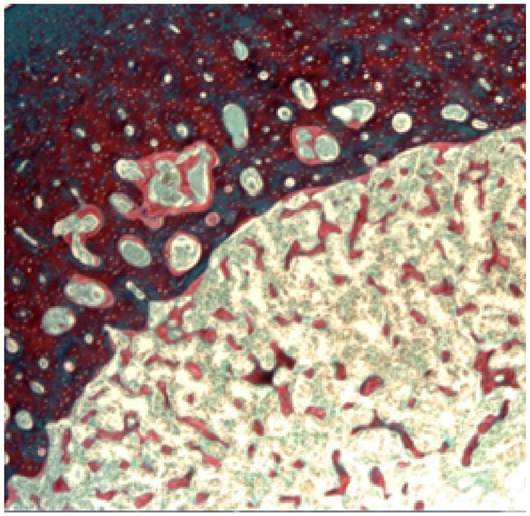
To characterize calcified tissues we need to use different analytical techniques to get information from:

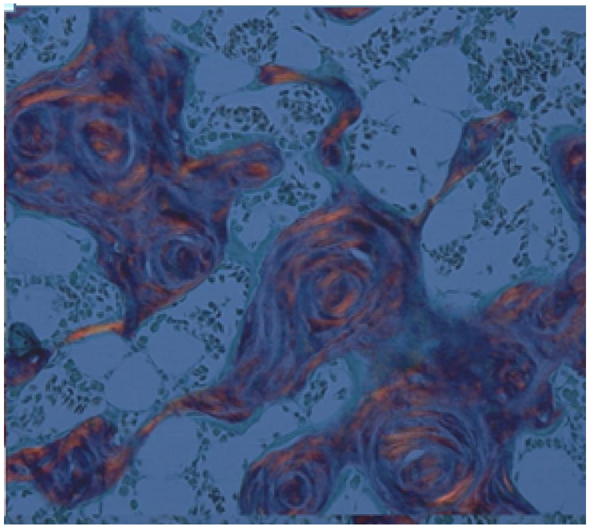
- **Constituting inorganic and organic components:**

Analytical techniques provide information at different levels of chemical and structural complexity:

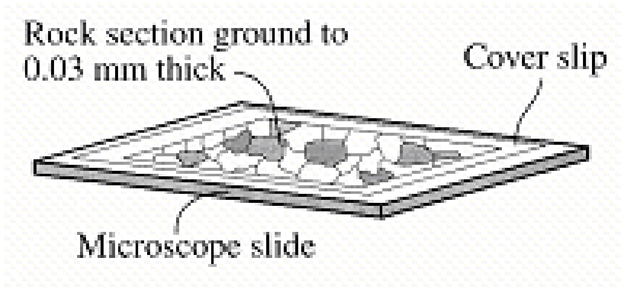
- **Elemental composition: ICP-OES, EDX (Ca, Fe, P, S, …)**
- **Molecular groups: IR, Raman spectroscopy (carbonates, phosphates, …).**
- **Mineralogical composition: XRD, TEM (calcite, aragonite, apatite,)**
- **Morphology, size and crystal orientation: OM, SEM, TEM, XRD, EBSD.**

Optical microscopy



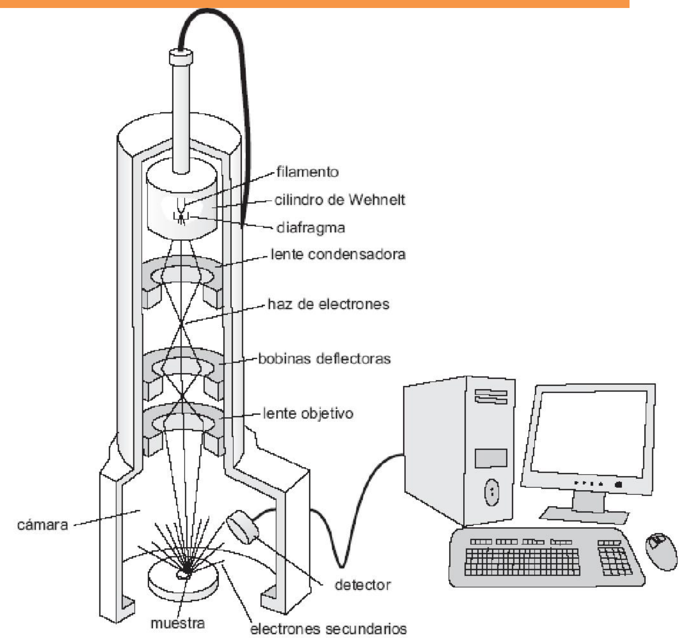


Parallel and cross-polarized light microphotograhs of hen bone

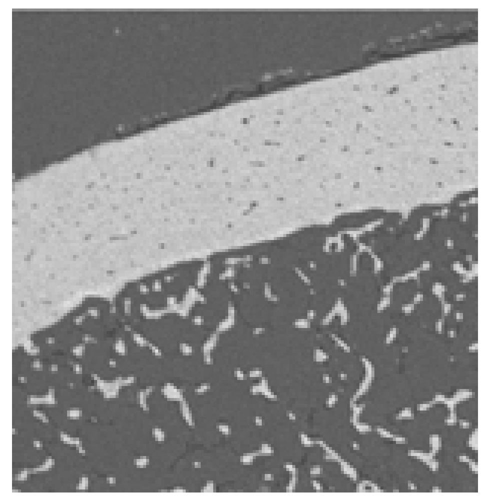


- *Very detailed information about the microstructure (crystal size, shape and orientation) and distribution of organic matter.*
- *Sample preparation is very tedious.*
- *Difficult quantification of parameters.*

Scanning electron microscopy



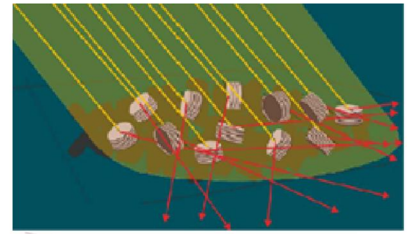
- SEM with different types of detectors (SE, BSE, EDX, EBSD) (Auriga, Zeiss).
- SE (topography), BSE, EDX (compositional information)
- EBSD electron diffraction – mineral/crystal orientation mapping.

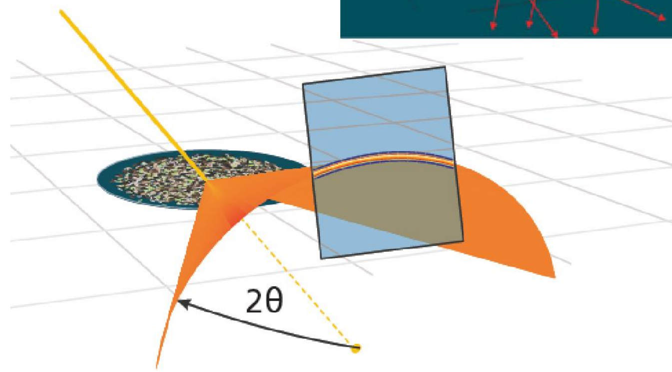


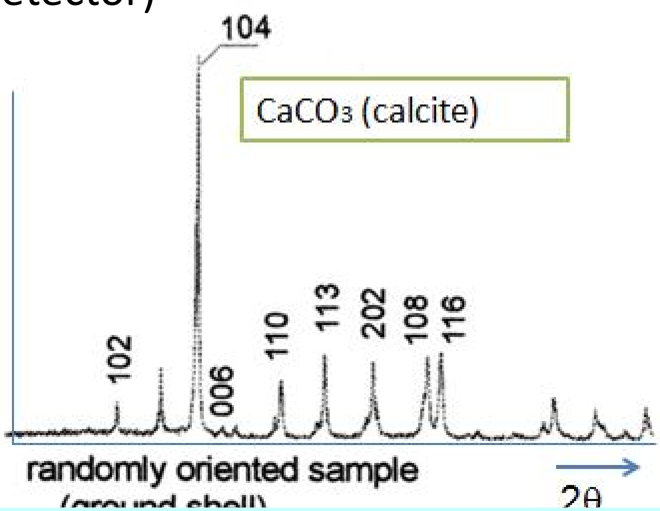
•BS images of a hen bone

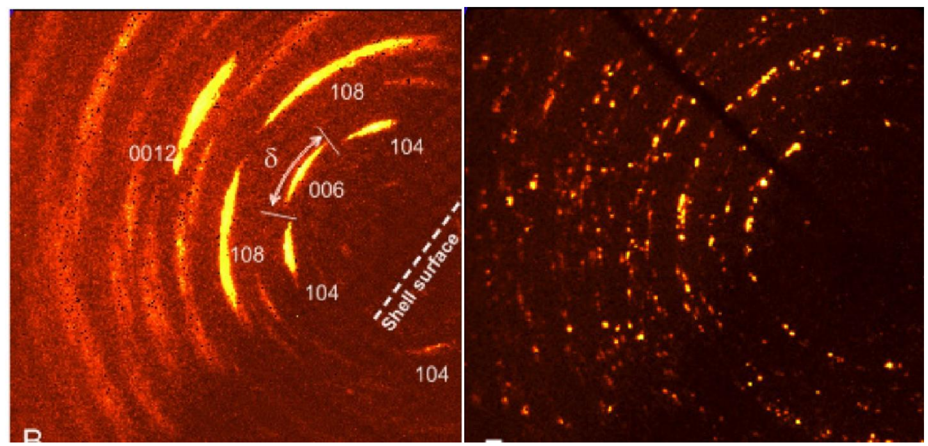
2D X-ray diffraction techniques

Single crystal diffractometer Bruker (SMART APEX detector)









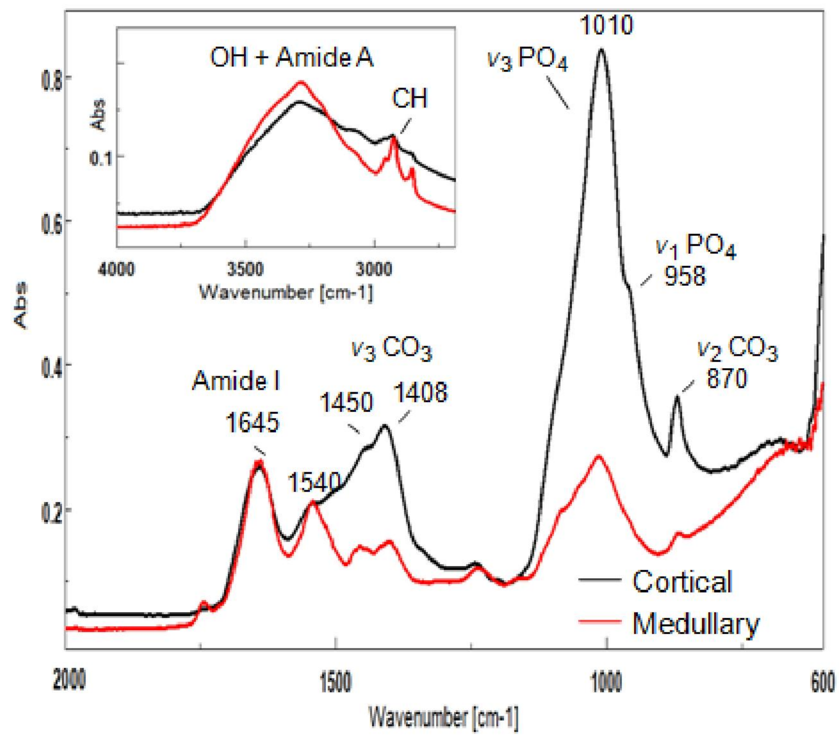
Crystals behave like little mirrors. Preferential orientation results in arcs formation

Conventional X-ray diffractometers are used for mineralogical composition analyses where as diffractometer with a 2D detector are used for microstructural information.

Infrared spectroscopy (IR)

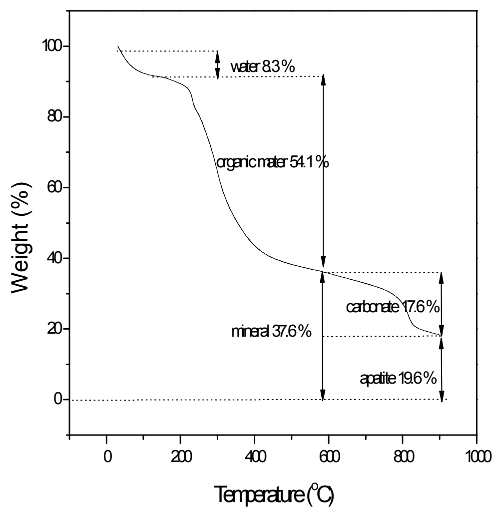
Fourier Transform Infrared Spectrometer (FTIR)

ATR-FTIR probes the chemical composition of the bone material.

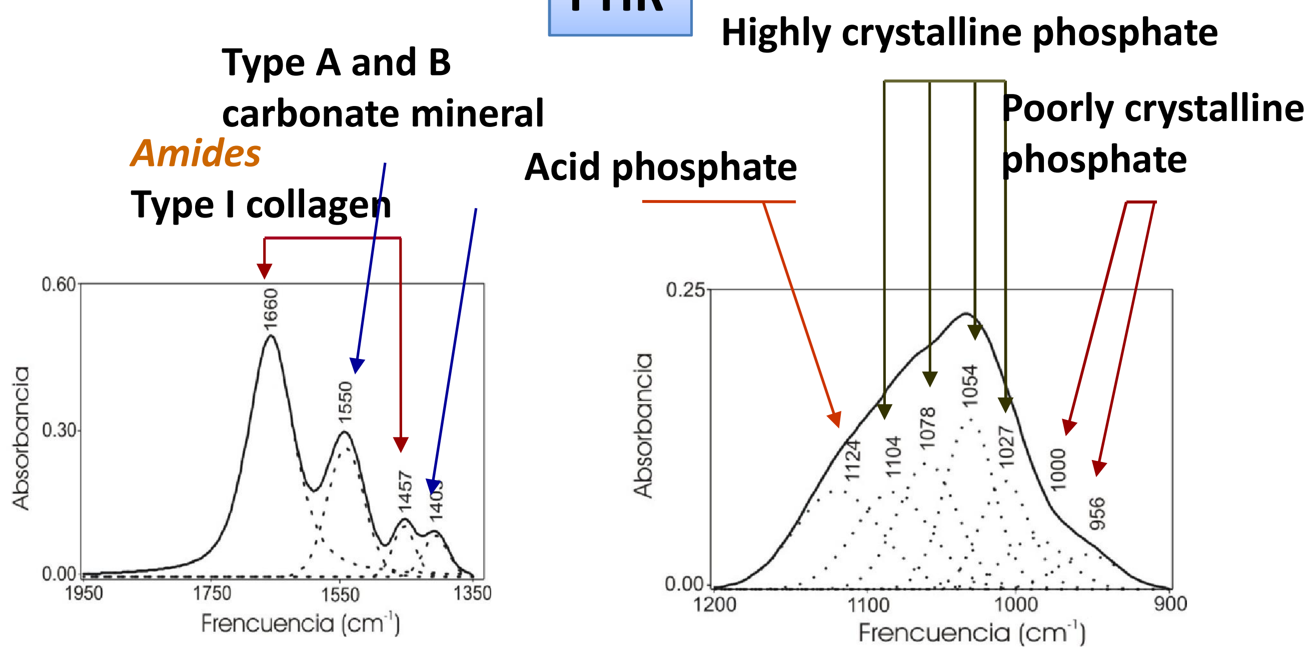


Chemical composition of bone

TGA



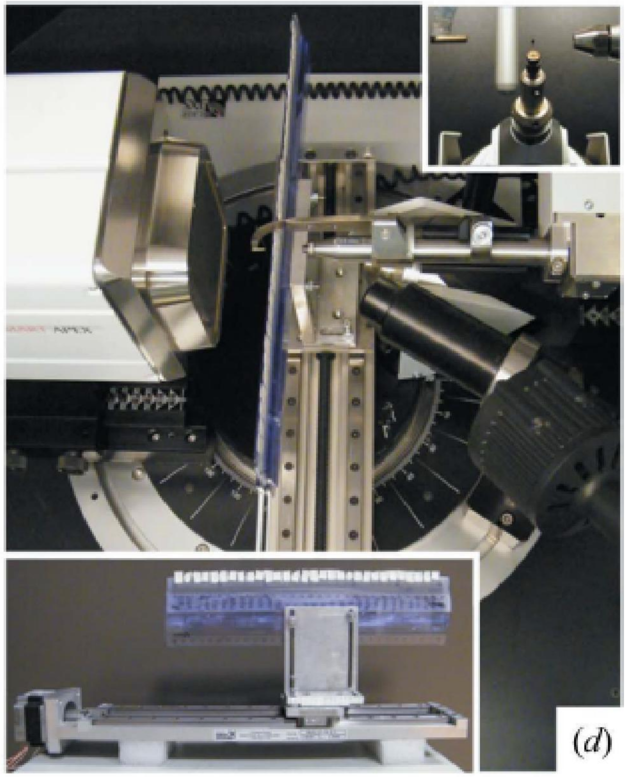
FTIR



Bone has a very complex chemical composition (mineral, nanocrystalline carbonated apatite, + organic matrix, mainly type I collagen).
TGA and FTIR analyses were used to determine bone composition.

Bone chemical composition changes with age and skeletal location. It is informative of bone metabolic status and bone health.

Development of analytical tools



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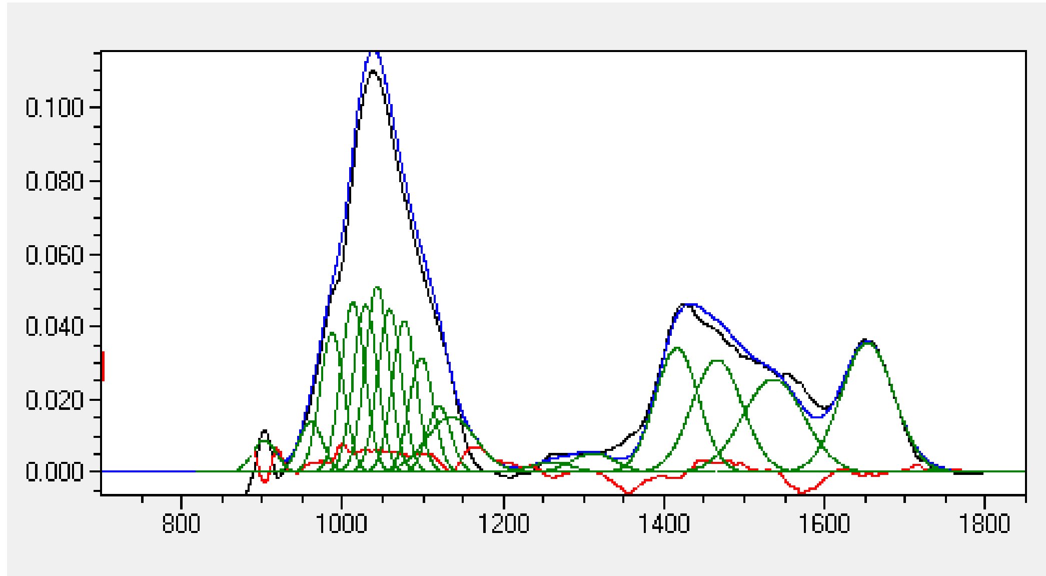
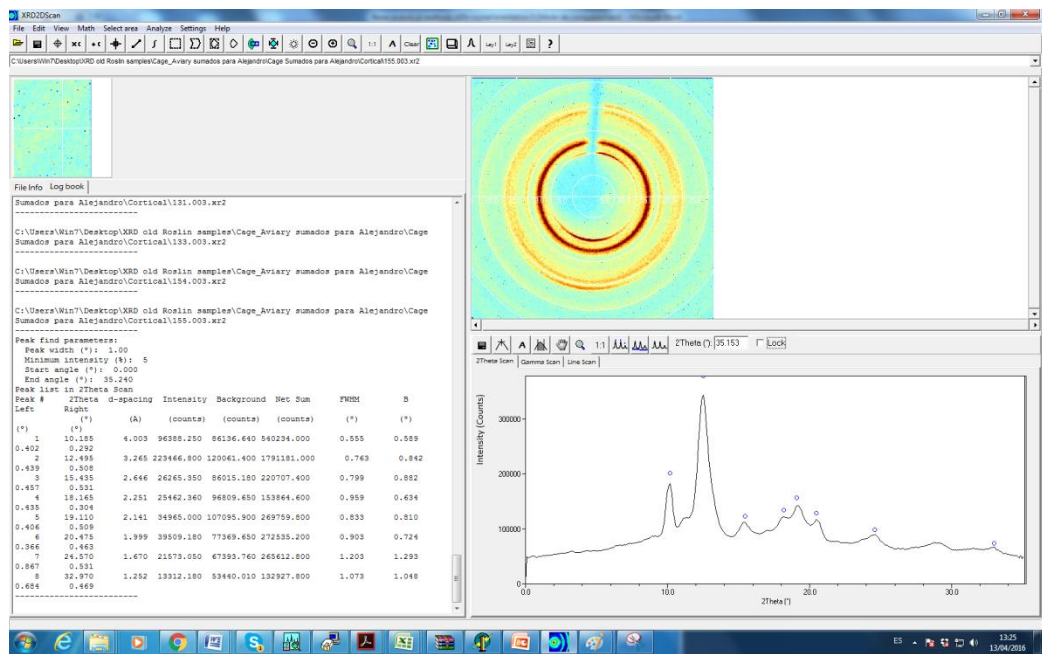
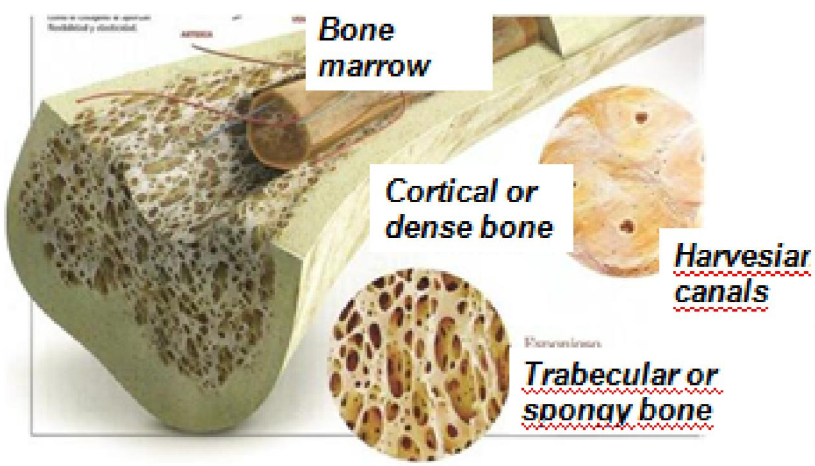


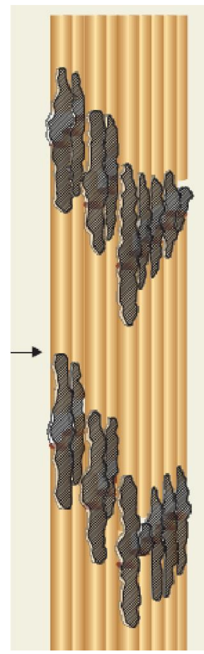


We have developed specific sample changer and software tools to automatically analyze a large number of samples and analyze FTIR and XRD data of bone samples more efficiently.

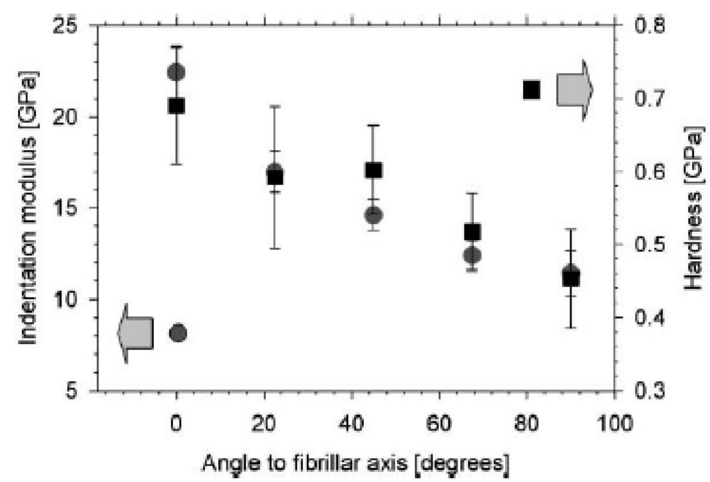
Bone mineral and collagen

Bone is a composite of apatite nanocrystals reinforcing an organic matrix of cross-linked collage fibres. **Highly organized at different scales => excellent mechanical properties**





Apatite crystal platelets are aligned to the collagen fibres.



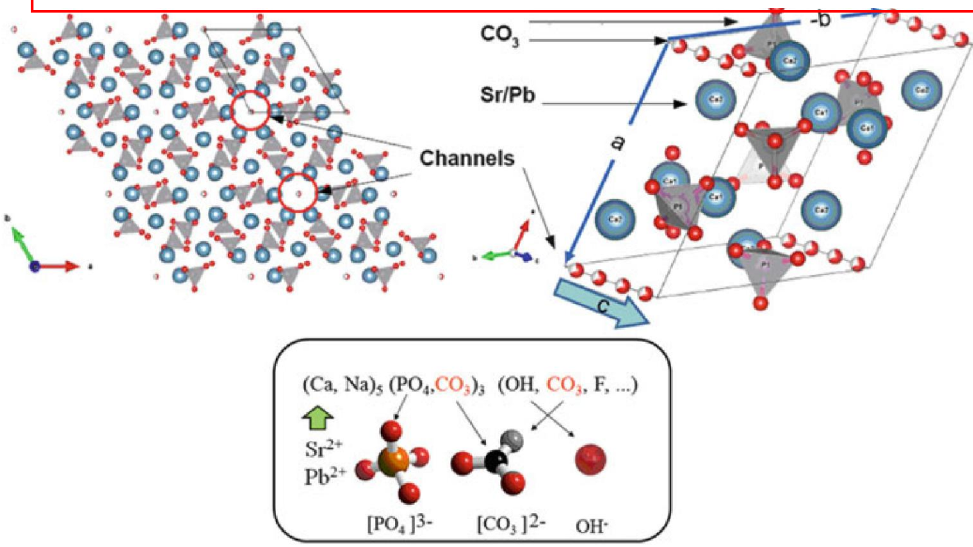
Bone mechanical properties (strength) is determined by bone mass, geometry and **bone quality**.

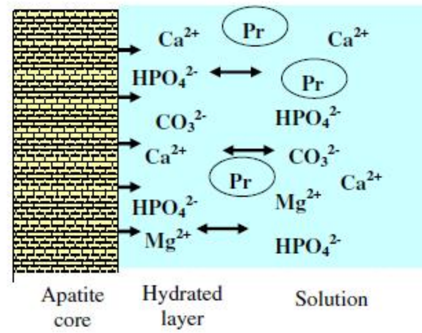
Bone quality is dependent on degree of mineralization, microarchitecture, microdamage, bone mineral and collagen composition. All these characteristics are dependent on turnover rate and tissue age.

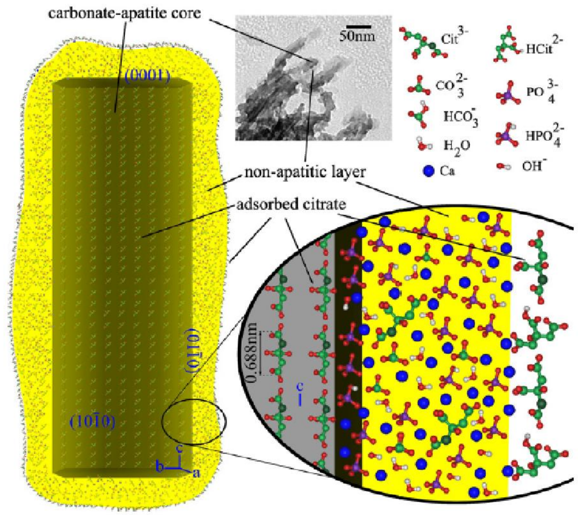
Fraztl et al., 2004; Compston 2006; Schmal et al., 2015

Bone mineral and collagen

Bone mineral composition and crystallinity changes with tissue age and/or turnover rate.







-Bone apatite crystals have a variable composition due to ionic substitution:

Ca^{2+} <-> Mg^{2+}, Na^{+}, Sr^{2+}

PO_4 <-> $CO_3{}^{2-}$ (Type B); OH - <-> $CO_3{}^{2-}$ (Type A).

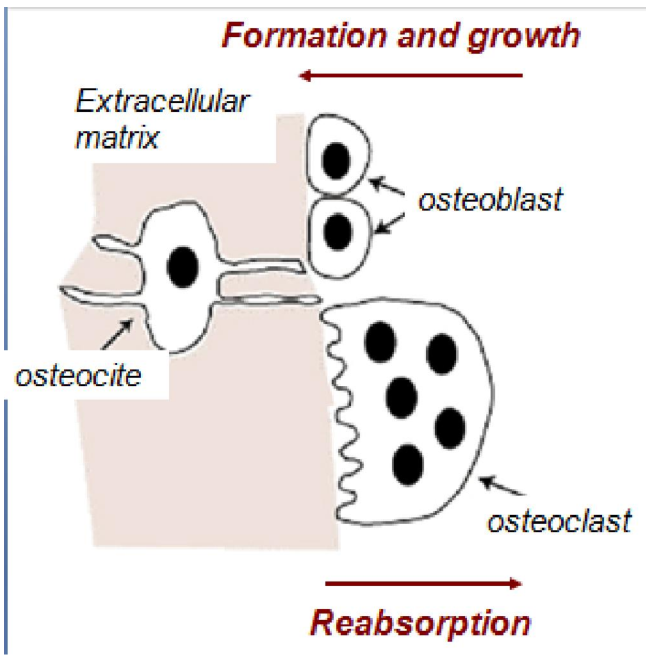
-There are labil forms of carbonate (more soluble), and citrate absorbed on the crystal surface.

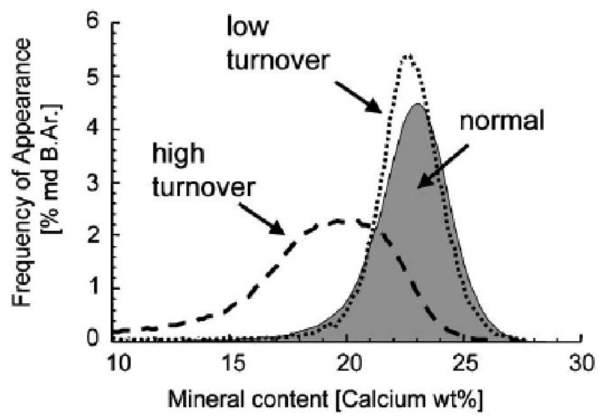
Rey and Glimcher 2009 Osteoporosis Int.; Delgado-Lopez et al., 2012 Acta Biomaterialia

Schmal et al., 2015

Bone mineralization and remodelling

Bone is a living organ in constant remodelation by bone forming (osteoblast) and resorving (osteoclast) cells.
It also allows to heal and adapt to external mechanical loads.
Bone mineralization is controlled by a wide array of biochemical components (hormones, growth factors).





BMD distribution in healthy and disease bone.
Fratzl et al. JMatChem 2004

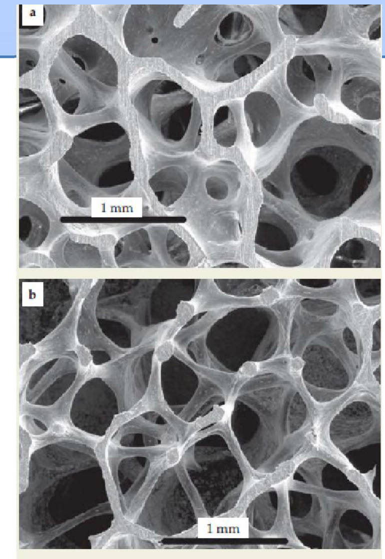


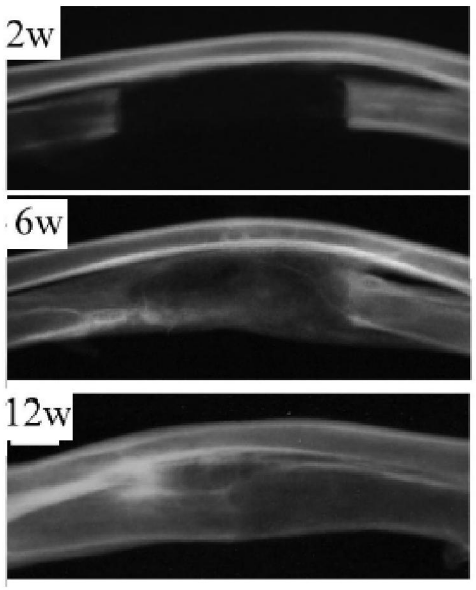
Figure 5. Spongy bone from inside vertebrae obtained

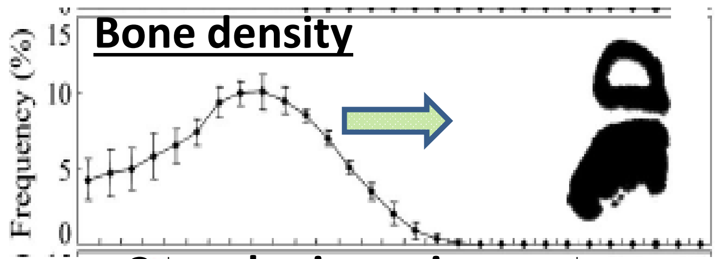
Healthy (top) and osteoporotic (botton) trabecular bone.
Ritchie et al. Physic Today 2009

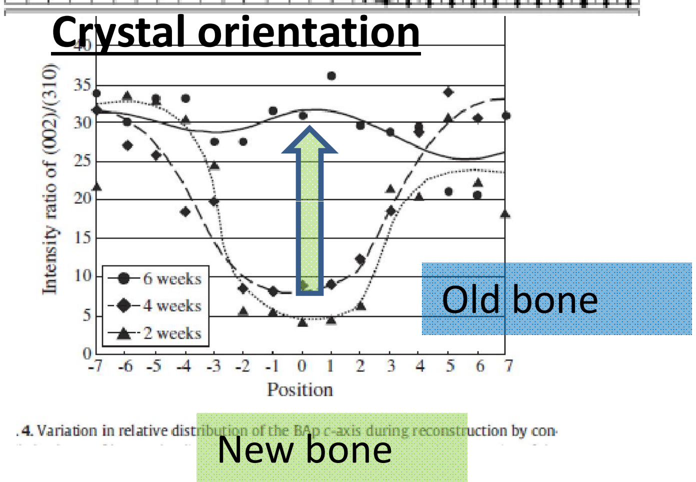
The development of different bone pathologies (osteoporosis) is due to an unbalanced bone mineralization and/or altered turn over rate.
Bone in health and disease has a characteristic mineral distribution and organization

Bone formation, healing and reorganization

Bone is a living tissue capable of self-healing. It can recover the original structure of the fractured bone



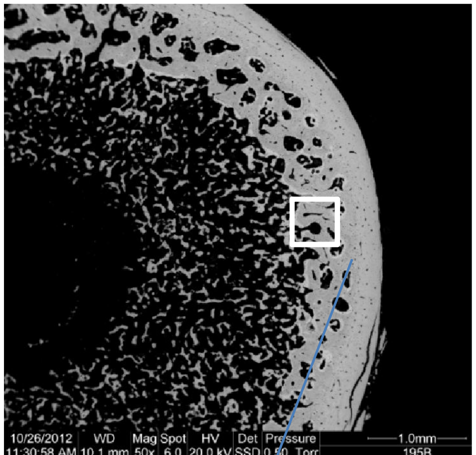


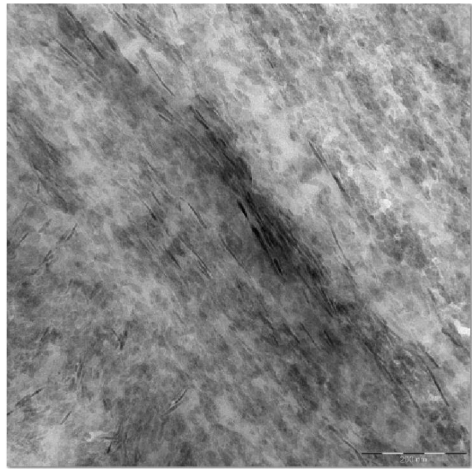


During **new bone formation or healing**, the bone mineral density gradually increases.
Mineral crystallinity and crystal orientation also increases though the later process is delayed.

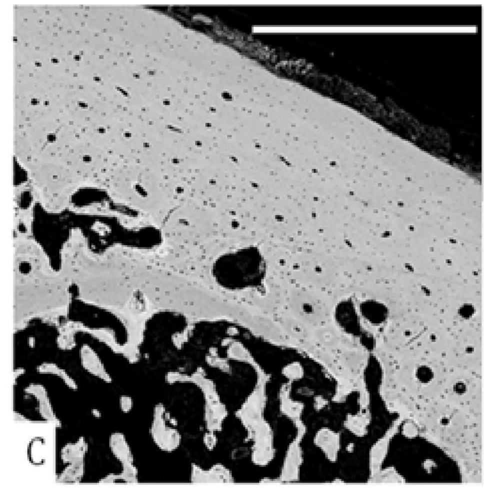
Ishimoto et al., 2013 JBMR;

Hen bone structure at the micro- nano- scale

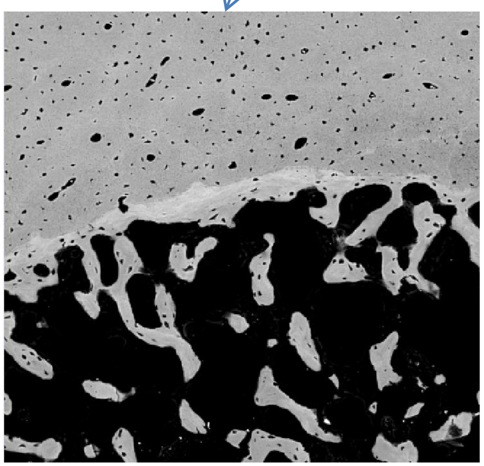


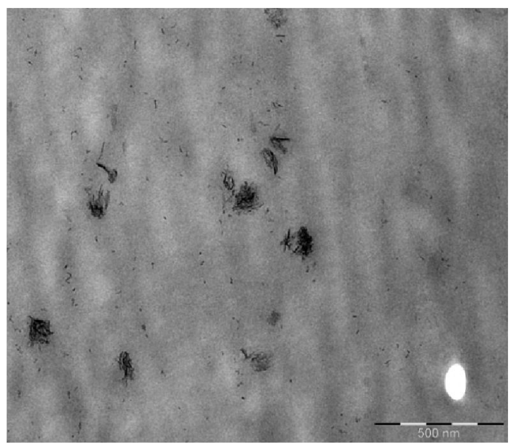


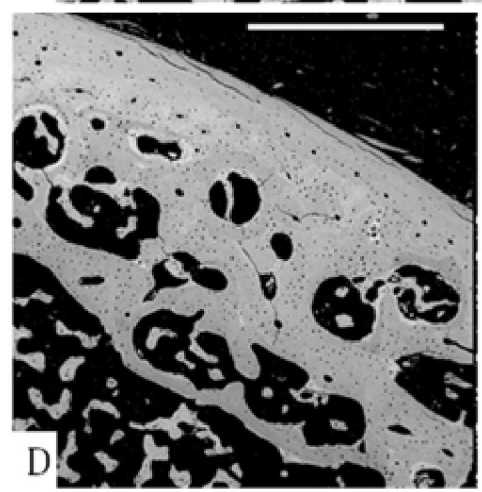
Strong bones



Cortical bone: Compact ,highly oriented crystals
Structurally strong



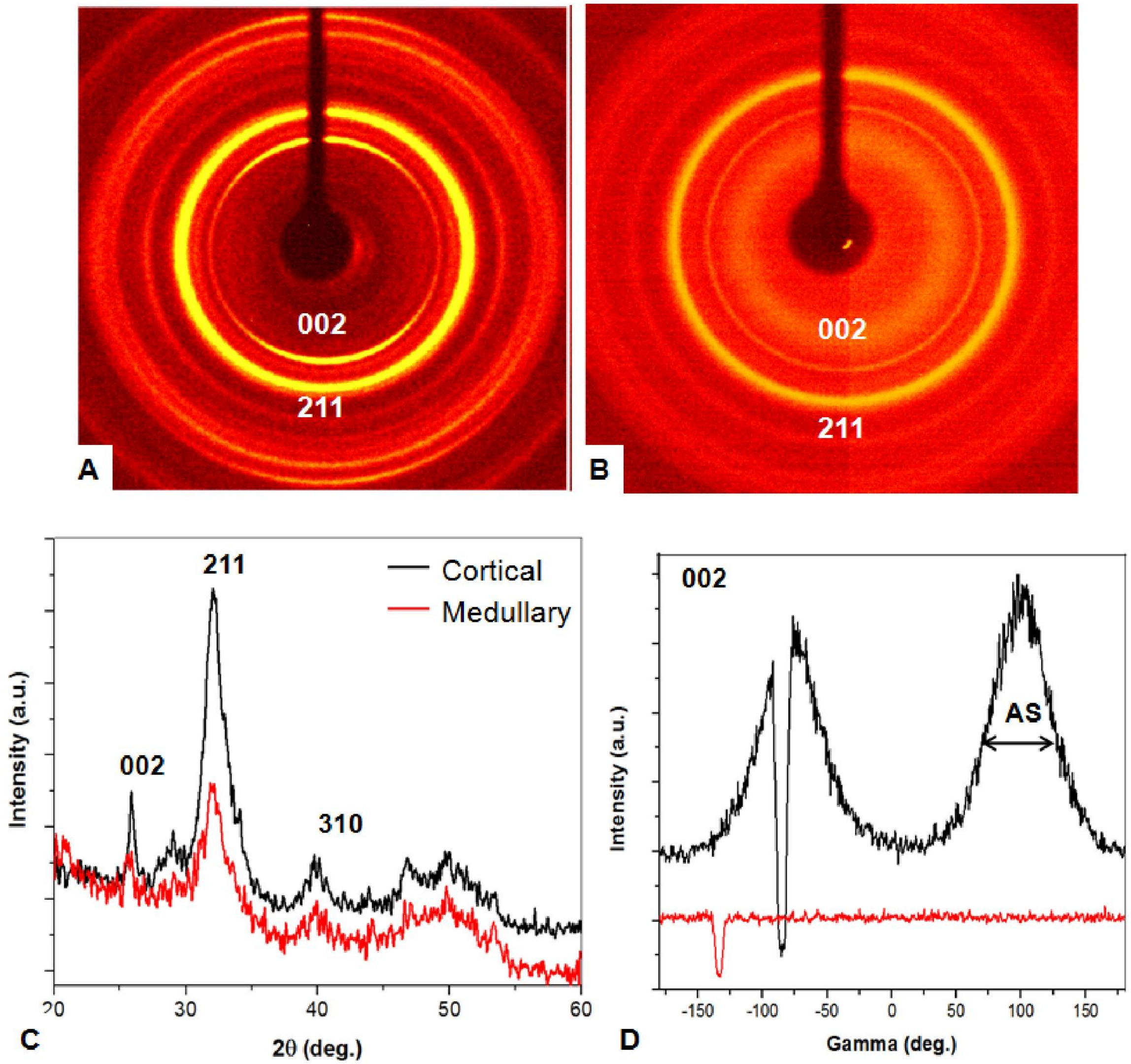


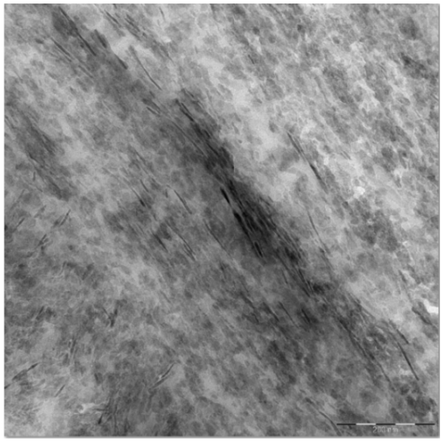


Medullary bone: isotropic , no crystal orientation
structurally weak

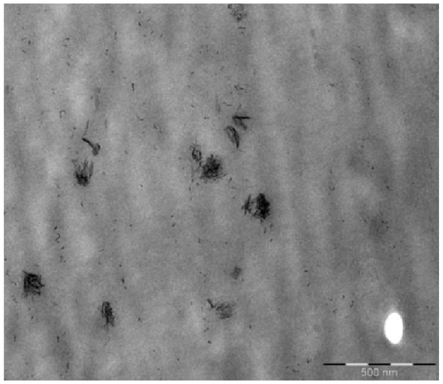
Weak bones

Crystallinity and crystal orientation of cortical and medullary bone mineral





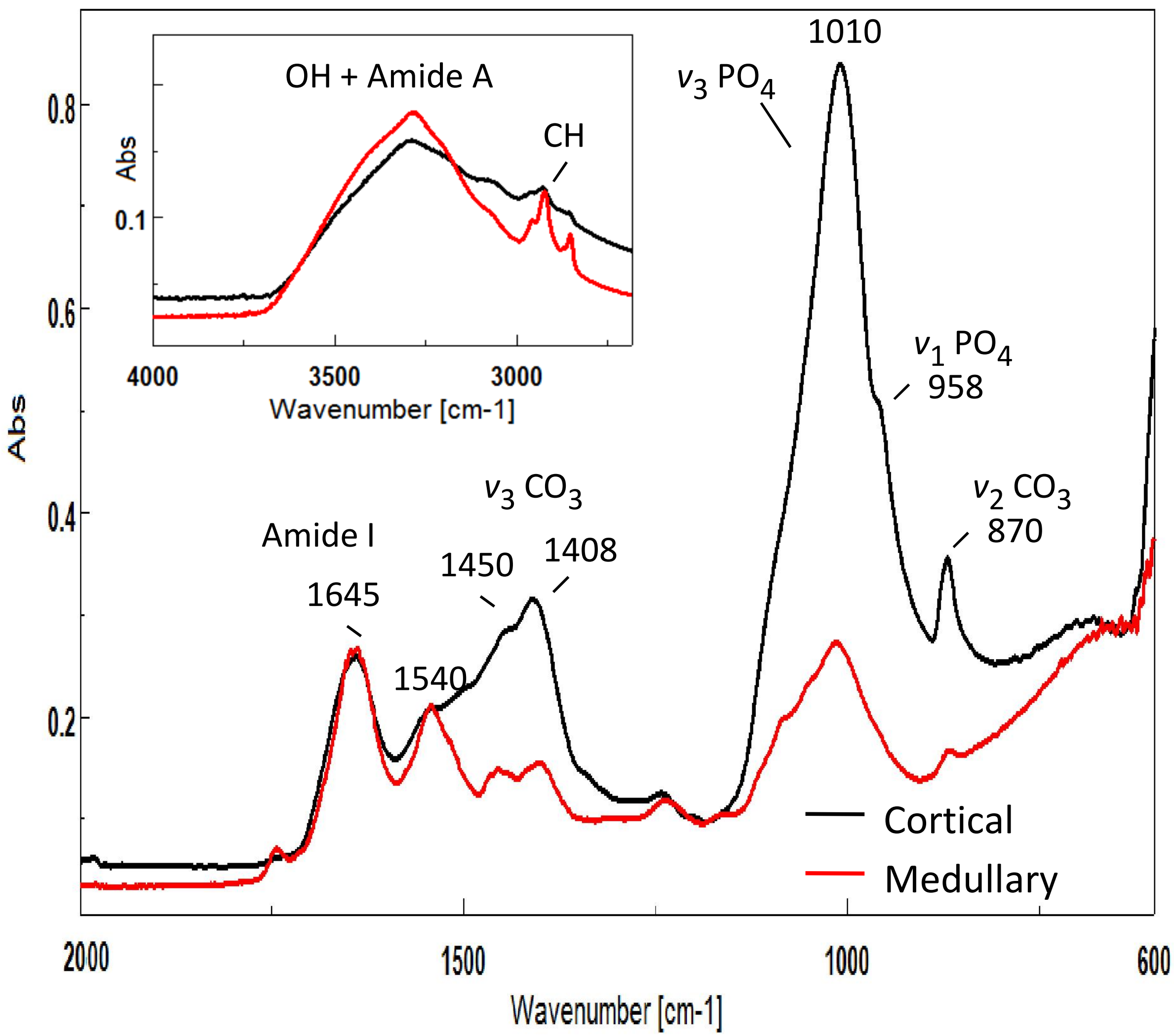
Cortical bone: (TEM)



Medullary bone: (TEM)

Cortical bone is more crystalline and apatite (and collagen fibres) are preferentially oriented parallel to the long bone axis.
Medullary bone mineral is less crystalline and isotropic (disorganized).

Chemical composition of cortical and medullary bone by FTIR



Cortical bone is more mineralized and the bone mineral is more mature (higher crystal carbonate) than that of medullary bone

Aknowledgements

- Dr. Heather McCormack, Dr. Robert Fleming, Dr. Ian Dun Roslin Institut, UK.
- Nazaret Dominguez-Gasca, Pedro Alvarez Lloret, Annika Parviainen, UGR, SPAIN.
- Bendición Funes, José Romero Garzón, , CIC, UGR, Spain.
- Funding from Roslin Institute, projects CGL2011-25906 (MEyC, Spain) and Better Bones (ERA-Net ANHIWA).