A quantitative study of porosity classes in trabecular bone by MR-Relaxation and MR-Cryoporometry

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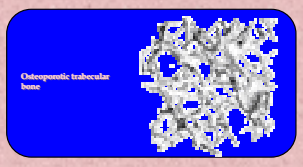
ABSTRACT

The interest in Magnetic Resonance studies of bone is increasing as new noninvasive MR Imaging methods are being developed to study bone microstructure and the effects of diseases such as osteoporosis. In a preliminary study[1], the feasibility and potential for the combined use of MR-Relaxation (MRR) of ^{1}H nuclei and MR-Cryoporometry (MRC)[2] for the study of bone microstructure was demonstrated. The study has been extended to the quantitative characterization of particular classes of pore spaces in trabecular bone, with a special emphasis on the intratrabecular space where MRC is expected to be more sensitive. It has been shown[3] that from MRR it is possible to distinguish between the signal of water in the large inter-trabecular spaces and that in the smaller intra-trabecular spaces. This work provides solid validation of those results with the characterization of different structures in the bone pores for a large set of samples. MRC offers evidence for the existence of pores with diameters less than 4000Å in bone. MRR analysis allows one to distinguish and quantify the fraction of pore volume in the inter- and intra-trabecular spaces in such a way that the bone volume fraction (BVF) can be determined. BVF is defined as the ratio of solid bone volume (including any intra-trabecular pore space) to total bone volume (including all pore space); it is a parameter usually determined by histomorphometric analysis, quantitative 3D X-Ray or MRI microtomography. In addition, our combined magnetic resonance methods allow one to measure the internal porosity of the trabeculae (Φ_{trab}), defined as the ratio of intra-trabecular pore volume to the total volume occupied by the trabeculae.



RESULTS MR RELAXATION

Procedures were adopted[4] to subtract the contribution of macromolecular ^{1}H from the total signal (Fig1a,b). Therefore, in this paper only ^{1}H from water have been considered. Fig 2 shows quasi-continuous distributions of T_1 for 4 distal femur samples, representative of 4 different classes of samples identified. The main feature they have in common is the presence of two peaks, either resolved or partially resolved, with a small tail going down to about 1 ms, indicating some very small pores.What differentiates the curves is the signal fraction, that is particularly abundant in two of them for T_1 shorter than 1 s. Longer relaxation times are expected to reflect the confinement effect in larger spaces between one trabecula and another. Progressive drying has confirmed that the long T_1 components were, in fact, in the large intertrabecular spaces, as the corresponding peaks have been lost following the process[3]. Facing bimodal distributions gives one a clue to assume a cutoff in correspondence of the minimum of each curve, so that their intra- and inter-trabecular spaces can be easily separated and the corresponding signals quantified. The ratios of inter-trabecular and intra-trabecular pore volumes to total pore volume can be evaluated as $S_{inter}/(S_{inter}+S_{intra})$ and $S_{intra}/(S_{inter}+S_{intra})$, respectively. From these data BVF can be calculated as $BVF=1-\Phi[S_{inter}/(S_{inter}+S_{intra})]$, where Φ is the total porosity. The latter is determined by weighing the sample before and after water saturation. From these parameters the value of Φ_{trab} can be straightforwardly computed (see table).

In the end it is worth pointing out that the BVF values obtained are consistent with data from other sources. In addition, all samples included, Φ_{trab} can be estimated of the order of 30%. We do not know of any other method to determine Φ_{trab} in a simple and non-destructive way.

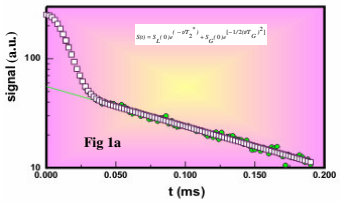


Fig 1a

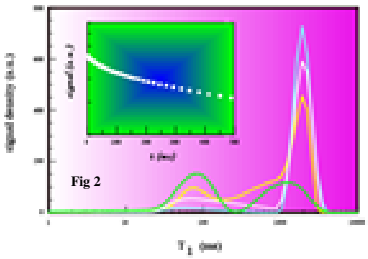


Fig 2

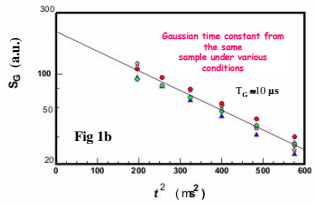


Fig 1b

Quantitative evaluation of bone volume fraction (BVF) and intra-trabecular porosity (Φ_{in})

N°	Φ(%)	$S_{intra}/(S_{inter}+S_{intra})$	$BVF=1-V_{inter}/V_{tot}$	$\Phi_{trab}=V_{intra}/(V_{min}+V_{intra})$
4	45.6	0.55	0.79	0.31
8	59.9	0.25	0.55	0.27
12	66.0	0.11	0.42	0.17
16	67.1	0.22	0.47	0.30

MATERIALS AND METHODS

In this study cylindrical samples (7 mm in diameter and in height for MRR and 4mm in diameter and 15 mm in height for MRC), were cored paired in different positions of one femur of a year old cow, stored in the slaughterhouse refrigerator immediately after the slaughter. Samples for MRR measurements were cleaned in order to remove the natural fluids inside, pumped for 24 hours to remove air, and then saturated with water. MRR measurements (Inversion Recovery sequence for T_1, and CPMG, TE= 600µs, for T_2) were performed at 20 MHz (0.47 T) and 20 °C by means of a Bruker Minispec P20 (Karlsruhe, Germany) equipped with NMR data station (Stelar, Pavia, Italy). Quasi-continuous distributions of relaxation times were computed by UPEN[4] . As for MRC, samples were dried before adding sufficient anhydrous cyclohexane under dry nitrogen atmosphere to slightly overfill them (melting point 6.5°C, purity of the cyclohexane used was about 99.5%).The glass tubes were sealed under vacuum to remove any dissolved oxygen and prevent evaporation and contamination. All measurements were taken by cooling down to –30°C initially and then measuring liquid signal intensity for increasing temperature up to 10°C, in order to prevent supercooling effects.

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RESULTS MR CRYOPOROMETRY

The fraction $S_{intra}/(S_{inter}+S_{intra})$ obtained by MRR is supported by the existence of pores with sizes less than 4000Å, evaluated by MRC. Recently[1,2], CMR has been successfully used to determine pore sizes distributions in the mesopore range (typically 3 to 100 nm). Therefore, in terms of classes of porosity, the method would be able to detect PLC – Lacunar-canalicular porosity (radius of the canaliculus of about 100 nm), and at least in part CA – Collagen-apatite porosity (about 10nm).The method relies on the fact that a pure material confined within a cylindrical pore exhibits a melting point that is depressed from the normal (bulk) melting point by a temperature difference that is inversely proportional to the pore diameter. According to the Gibbs-Thompson equation (see ref. 1,2 for more detail about the cryoporometry method):

$$\Delta T_m = T_m - T_m(x) = \frac{4\sigma T_m}{x \Delta H_f \rho} = \frac{k}{x}$$

where: T_m is the crystal bulk melting point, $T_m(x)$ is the melting point of a crystal of size x, x is the size of the pore or the formed crystal in the pore, r is the density of the crystal, s is the surface energy of the solid-liquid interface and ΔH_f is the bulk enthalpy of fusion. Fig 3a shows an example of a Melting Point Curve. The measured volume of liquid is recorded as a function of the melting point depression. In Fig 3b the pore size distributions plotted reveal the presence of pores with diameters less than 4000 Å, thus validating the quantitative results collected by means of the MRR technique.

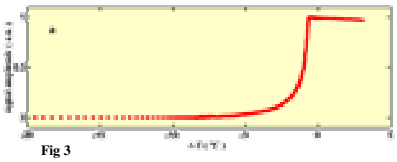
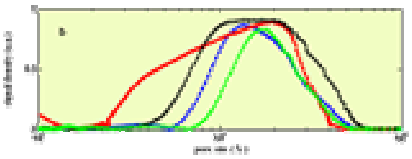


Fig 3



DIFFERENT LEVELS OF POROSITY IN BONE

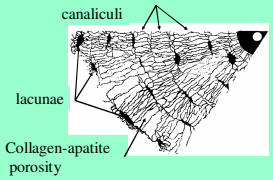
Quantitative analysis of the volume fraction of bone is being conducted frequently in the last decades. The ongoing exploration of structure-function relationships within affected tissue may provide insight into the mechanical and biological mechanism that lead to osteoporosis and other bone-related diseases. Literature reports on quantitative porosity measurements are sparse and mostly restricted to the vascular porosity of compact bone.According to Cowin[5] the levels of bone porosity can thus be classified:

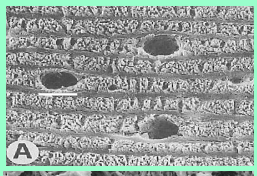
1. PV – Vascular Porosity: quasi-cylindrical passageways in the bone matrix (osteonal canals and Volkmann canals, of the order of 20 µm).

2. PLC – Lacunar-canalicular porosity: all the space in the lacunae and the canaliculi. The characteristic linear dimension associated with this space is the radius of the canaliculus (order 0.1 µm)

3. PCA – Collagen-apatite porosity: porosity associated with the spaces between the collagen and the crystallites of the mineral apatite (order 10nm radius).

4. PIT – porosity of the inter-trabecular space: the bone porosity with the largest, but quite variable, characteristic linear dimension (up to 1 mm) is associated with cancellous bone.





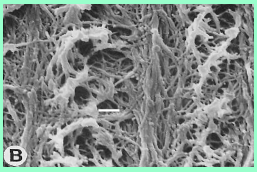


Fig A:SEM micrograph of human lamellar bone marker 10µm (example of PLC)
Fig B SEM micrograph of human osteon marker 1µm (example of PCA)
[ref: G. Marotti et al.Collagen texture and osteocyte distribution in lamellar bone.*IT.J.Anat.Embryol.Vol.100 Sup.n.1:95-102.1995*]