Diffusion behaviour of water and fat in bone marrow

Valentina Di Marco[1], Marco Palombo[1,2], and Silvia Capuani[1,2]
[1]*Physics Department, Sapienza University, Rome, Rome, Italy,* [2]*CNR IPCF UOS Roma, Physics Department, Sapienza University, Rome, Rome, Italy*

Purpose. Bone marrow is an heterogeneous complex system that consists of hematopoietic tissues islands and adipose cells surrounded by vascular sinuses[1]. In particular, bone marrow in the extremity of all the mammalian bones fills pores of the cancellous bone (trabecular bone marrow), while bone marrow located inside the central cavities of long bones is free, i.e. is not forced in pores. From a microstructural point of view both trabecular and free bone marrow are soft tissue, mainly characterized by different relative percentage of water and fat and comprised of several particles of spherical/elliptical shape and average size ranging from 6 μm of red blood cells to approximately 100 μm of the fat globules. Water in cancellous bone is more prevalent in the boundary zone while fats are rearranged primarily in the central zone of each pore[2]. Because the susceptibility mismatch between solid bone and bone marrow, bone marrow water in cancellous bone is affected by strong internal magnetic field gradients (G_i)[2]. G_i, are responsible of a loss of coherence that is faster as compared to that due to the usual spin-spin interaction and surface effects. Moreover, a G_i due to the magnetic difference between fat and water is also present and molecular dynamics associated with fat protons is much slower than that of water. Indeed, the apparent diffusion coefficient (*ADC*) of fat component in bone marrow is approximately two orders of magnitude lower than that of water molecules[2]. As a consequence, bone marrow water, that diffuses in the interstitial space between solid bone and fat, is influenced by the microstructures at the boundary of each pore and by G_i. NMR diffusion techniques have been widely used to investigate in a non invasive way the microstructural features of complex media. The aim of the present study was to investigate the potential of Gaussian and non-Gaussian diffusion methods to obtain information about the microstructural complexity and the water compartmentalization in free and trabecular bone marrow by investigating the water and fat *ADC* behaviour as a function of diffusion time.

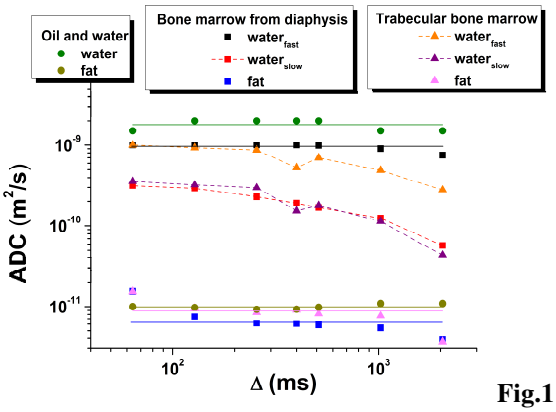

Fig.1

Methods. *Theory.* In pulse field gradient (PFG) diffusion measurements, the signal is typically recorded by diffusion-sensitized sequences as a function of chosen b-values range, where b includes both diffusion time Δ, diffusion gradients strength g and diffusion gradient duration δ: $b=(\gamma g\delta)^2\Delta$, with γ the ^{1}H gyromagnetic ratio. *ADC* can be extracted using Stejskal-Tanner equation which describes the signal behaviour as a function of b, as a simple exponential decay. However, the simple exponential decay inherently assumes diffusion to be Gaussian which means that there are neither obstruction nor irregular and chaotic travel paths of water molecular displacement. This is clearly an oversimplification in biological tissues as demonstrated by some experiments performed in the last few years which underlined the inadequacy of the simple exponential curve to fit diffusion data acquired in tissues characterized by an intrinsic microstructural complexity[3]. Several approaches have been proposed including the so-called anomalous diffusion (AD) stretched exponential model, in which γ is the stretching parameter arising from fitting the stretched function $S(b)=S(0)\exp(-(bADC)^{\gamma})$ to PFG data. Recent papers highlighted the ability of γ parameter to discriminate between different brain structures on the basis of their microstructural complexity[4,5] and their differences in G_i, at a microscopic scale[6]. In a bone marrow system we expected to measure two distinct pool of diffusing water molecules, fast and slow, corresponding to the extra and the intra cellular compartment, respectively, plus the fat protons diffusion pool. As a consequence, the diffusion PFG signal attenuation is expected to follow a multiple exponential decay: $S(b)=f_{fast}\exp(-ADC_{fast}b)+f_{slow}\exp(-ADC_{slow}b)+f_{fat}\exp(-ADC_{fat}b)$ [1], where ADC_{fat}, ADC_{fast} and ADC_{slow} are the *ADC* of the fat, the fast and the slow diffusing water, respectively, and f_{fat}, f_{fast} and f_{slow} are the fraction of fat molecules, fast and slow water diffusing molecules, respectively. However, because of the presence of G_i in trabecular and free bone marrow, we hypothesized that the PFG signal attenuation due to the fast extracellular diffusing water may be better characterized by a stretched exponential decay with respect to the simple exponential decay (the first term of relation [1]) and then we also tested $S(b)=f_{fast}\exp(-(ADC_{fast}b)^{\gamma})+f_{slow}\exp(-ADC_{slow}b)+f_{fat}\exp(-ADC_{fat}b)$ [2] relation. *Experiments.* We analysed samples of oil and water (as reference sample in which Gaussian diffusion occurs) and in vitro trabecular and free bone marrow extracted from calves, using a Bruker Avance spectrometer operating at 9.4T. A variable g Pulse Gradient STimulated Echo (PGSTE) sequence, with: *TE/TR* = 5/5000 ms, Δ = 64,128,256, 400,512,1024 and 2048ms, δ=4.4ms and 64 gradient amplitude steps from 1.2 to 120Gauss/cm (the corresponding b values run from $1{,}24\cdot10^7$ to $3.51\cdot10^{12}$ s/m^2) was used to extract ADC_{fat}, ADC_{fast} and ADC_{slow} values and f_{fat}, f_{fast} and f_{slow} values for all the three type of samples by fitting relation [1] to the acquired data. Moreover, relation [2] was also fitted to the acquired data to extract γ values for all the samples.

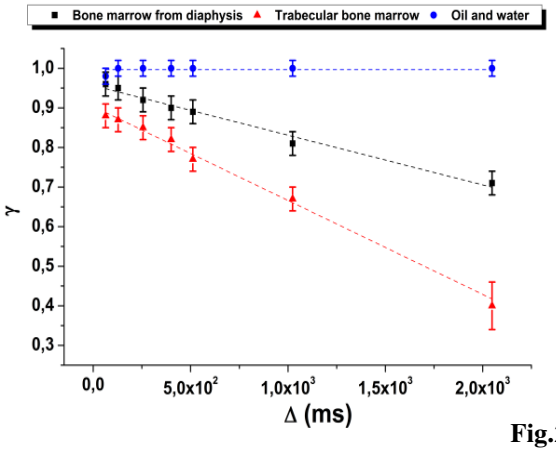

Fig.2

Results. The three *ADC* values corresponding to the three type of samples as a function of Δ obtained by fitting relation [1] to the PGSTE data are shown in **Fig.1**. In particular, only the ADC_{fast} and ADC_{fat} values are present in sample comprised of water and oil. The solid lines in **Fig.1** represent the constant mean *ADC* values while the dashed lines represent the trend of the time dependent *ADC* values. The γ values associated to the water in the three samples as a function of Δ obtained by fitting relation [2] to the PGSTE data are displayed in **Fig.2**. The dashed lines represent a linear relation fitted to the data from each sample.

Discussion. As expected, the control sample showed two diffusion components only, with *ADC* vs diffusion time constant, while free and trabecular bone marrow samples exhibit three diffusion components with different *ADC* values (**Fig.1**). In particular, in free bone marrow *ADC*s of the fat and of the fast extracellular water were found to be constant as a function of Δ, while the slow intracellular water exhibits a decreasing *ADC* value as a function of Δ. This behavior is typical of restricted water diffusion in enclosing geometry (the intracellular compartment). On the other hand, in trabecular bone marrow only the ADC_{fat} was found constant, while both ADC_{fast} and ADC_{slow} exhibit a decreasing trend as a function of Δ. This is coherent with the geometrical restrictions of the system: the water restricted in cells and the extracellular water between the fat and the bone in each pore of the cancellous bone. The differences between the extracellular water diffusion in the three type of samples are more evident in **Fig.2**, in which the γ values have a different behavior as a function of Δ in each type of sample. In particular, γ is constant (equal to 1) at all Δ values in control sample, while γ values decrease parallel to Δ values increase in free and trabecular bone marrow samples. Since in trabecular there are stronger G_i with respect to the free bone marrow, γ values are lower in trabecular than in free bone marrow at all Δ values.

Conclusion. In this work we highlighted the potential ability of Gaussian and non-Gaussian water diffusion in bone marrow to characterize and discriminate different bone marrow samples. Experimental results shown that γ is highly correlated to intrinsic local features at the interface between water and bone.

References. [1]Travlos GS. Normal structure, function,and histology of the bone marrow. Tox Pathol 2006;34:548-565. [2]De Santis S, Rebuzzi M, Di Pietro G, et al., In vitro and in vivo MR evaluation of internal gradient to assess trabecular bone density. Phys Med Biol 2010;55(19): 5767-5785. [3]Palombo M., Gabrielli A., De Santis S. et al., Spatio-temporal anomalous diffusion in heterogeneous media by NMR, J. Chem. Phys. 2011; 135(3):034504-034511. [4]De Santis S., Gabrielli A., Bozzali M., et al., Anisotropic anomalous diffusion assessed in the human brain by scalar invariant indices, Magn. Reson. Med. 2011;65(4):1043-1052.[5]Palombo M., Gabrielli A., De Santis S. et al., The γ parameter of the stretched-exponential model is influenced by internal gradients: Validation in phantoms, J. Magn. Reson. 2012;216:28-36.