Correlation of osteoid water diffusional transport properties with porosity of cortical bones

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Introduction

Water plays a critical role during mineralization and as a carrier of nutrients and waste products to and from osteocytes, previous study has suggested that NMR imaging and spectroscopy based on monitoring the H_2O/D_2O exchange kinetics is uniquely suited for detailed insight into the diffusional transport mechanisms across the bone matrix. In this work, in order to support the hypothesis that there is a close relationship between osteoid water diffusion coefficients with bone porosity and that this effect can be quantified nondestructively by NMR, an animal model of osteomalacia was studied. Positive correlation of diffusion coefficient of osteiod water in cortical bones measured by NMR spectroscopy with porosity measured by high resolution micro-CT data was revealed.

Materials and Methods

Two groups of 5-6 week old New Zealand White rabbits (N=5 in each) were studied: Group I were control animals receiving normal diet, and Group II received a hypophosphatemic diet for the same period of 8 weeks. Afterwards the animals were sacrificed and the whole bone specimens were harvested and placed into the refrigerator at -20^{o}C until experiments were done. Water diffusion coefficient: The NMR experiments were performed using a vertical-bore superconducting spectrometer system, operating at 9.4T (DMX-400, Bruker Instruments) in a 5mm proton probe. The samples were rectangular section (10×4×1mm) of cortical bones, with the marrow removed, harvested from the posterior side of mid-shaft of the right tibias. Upon removal from storage in saline (to prevent dehydrating), surface water was removed by brief padding with tissue paper. Water diffusion coefficient from the bone matrix was quantified by measuring the volume of H2O displaced after immersion of the bone in 0.6ml of deuterium oxide (D_2O, 99.9% isotopic purity, Sigma Chemical Co., St. Louis, MO) using proton NMR at 25^{0}C. Diffusion coefficient was calculated as

$$I(t) = I(\infty)\frac{4}{\pi^{\frac{1}{2}}}\left(\frac{D_a t}{d^2}\right)^{\frac{1}{2}} \quad (1)$$

in which $I(\infty)$ is the integrated signal intensity at equilibrium, D_a is the apparent diffusion coefficient of water through the bone matrix, d is the specimen thickness, and t is the exchange time at initial stage(within 5minutes). Porosity: Approximately 4 cm long pieces were cut from the mid diaphysis of left tibias of the corresponding rabbit. Three-dimensional images were obtained using an EVS MS-8 micro-CT system (EVS-GE Medical Systems) at voxel resolution 8µm x 8µm x 8µm. Porosity is defined as lacunae volume divided by the total volume occupied by bone mineral and lacunae.

Results and Discussion

Figure 1 shows evolution curves of the water proton NMR signal for cortical bone specimens suspended in D2O at 25^{o}C

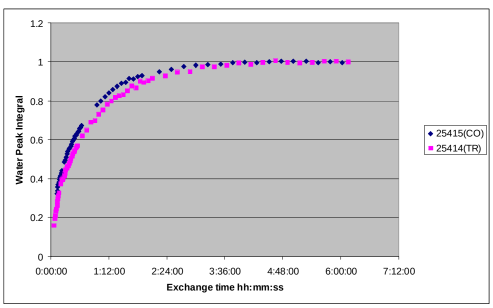

Table 1 shows the data of ten specimens in control and treatment groups

Animal	Diffusion Coefficient (mm2/s)	Porosity (%)
25461(CO)	2.53E-07	0.51
25462(TR)	3.90E-07	2.91
25463(TR)	2.92E-07	0.94
25464(CO)	2.40E-07	0.94
25412(TR)	8.76E-07	1.77
25413(CO)	4.75E-07	0.18
25414(TR)	5.67E-07	1.86
25415(CO)	4.43E-07	0.62
25416(CO)	4.83E-07	0.82
25417(TR)	2.87E-07	2.21
	Mean ± Std	Mean ± Std
CO	(3.79 ±1.22)E-07	0.61±0.29
TR	(4.82± 2.48)E-07	1.94±0.72

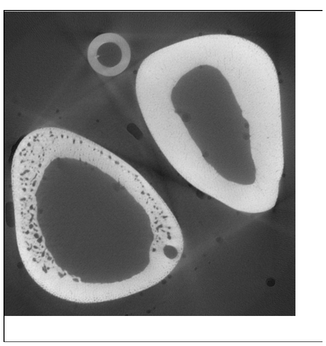

Figure 2 shows a slice image from µCT of a control and a treatment specimen at resolution 8µm by 8µm, where porosity is calculated by thresholding intensity to find pore area and bone mineral area.

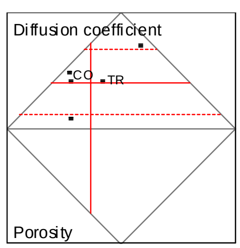

Figure3

Diffusion coefficient	5.296	t-Ratio	3.92048928
Porosity	1.328	DF	4
Mean Difference	3.968	Prob > |t|	0.01724057
Std Error	1.01211857	Prob > t	0.00862029
Upper95%	6.77805363	Prob < t	0.99137971
Lower95%	1.15794637		.
N	5		.
Correlation	0.10840551		

Conclusion

The diffusion coefficient of the solute in the fluid that fills the pores is determined by the interaction between solute molecules and matrix, the actual length of the diffusion path, and the matrix porosity. Water diffusion coefficient during the exchange of D_2O/H_2O could be indications of the degree of porosity of the matrix , assuming that the interaction between the water molecules and the channel walls does not significantly affect diffusion, which is a sensible assumption for molecules that are small, compared with the pore size (2).

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