CORRELATION BETWEEN SEM MEASURED MICROSTRUCTURE AND NMR PREDICTED BONE POROSITY

Yixian Qin (1), Hoyan Lam (1), Clint Rubin (1), Fred Grine (2), Qingwen Ni (3)

Department of Biomedical Engineering
SUNY Stony Brook
Stony Brook, New York

Department of Anthropology
SUNY Stony Brook
Stony Brook, New York

Southwest Research Institute
San Antonio, Texas

INTRODUCTION

The motion of interstitial fluid in bone, which arises as a result of functional loading, is hypothesized to be a critical mediator in the perception and response of skeletal tissue to mechanical stimuli [1, 2, 3]. Fluid movement in mature osteonal bone has been studied both theoretically and experimentally [1,4]. Despite the inevitably complex characteristics of fluid flow in porous media, bone fluid flow driven by loading represents and ideal "transducer" of the mechanical load to the adaptive response of bone, particularly as it is so well coupled with strain magnitude, rate, gradients, load distribution, as well as nutrition supply [1,5].

Interstitial fluid fills the various macro and micro porosities, osteon, lacunae, and intercellular channels in bone, including the canaliculi, Haversian canals, and even micro pores [3]. Despite the inevitably complex characteristics of fluid flow in porous media (e.g., time and pressure gradient dependent fluid movements), bone fluid flow driven by loading may be necessary to explain the adaptive response of bone, which is coupled with load-induced strain magnitude or independent with matrix strain *per se*.

Defining the interstitial fluid path through the porous bone structure will help improve our understanding of physical stimulation associated with adaptation through mechanical strain and fluid shear stress on the cellular response. The porosity and its associated fluid flow pathways, however, must be defined before we can determine the physical basis of mechanotransduction in bone. While it was demonstrated to be extremely difficult to measure spatial distributions of intracortical fluid components in vivo and experimentally, the porosity will be non-invasively evaluated using nuclear magnetic resonance (NMR) technique. NMR represents a new modality which can potentially serve as an efficient tool for determining bone pore size, an essential parameter for analyzing intracortical fluid flow analytically and experimentally. The objective for this study is to measure in vitro bone porosity using NMR and validate this with a histomorphometric method, i.e., high resolution backscatter electron microscopy (SEM).

METHODS

Bone samples preparation: The cortical bone of the mid-diaphyses of the ulnae of 1-year-old male turkeys was dissected from freshly slaughtered animals. These samples are categorized from normal (N=4) and 4-week disuse treated by functionally isolated osteotomies (N=4). The bone samples were cut 2-cm long using a diamond saw with saline solutions. The soft tissue attachment was carefully cleaned up from endosteal and periosteal surfaces.

NMR measurements and determination of porosity: The test of NMR was performed at the Southwest Research Institute (SwRI), where a SwRI built 0.5 to 40 MHz broad-line NMR was configured for a proton frequency of 2.3 MHz for these measurements. A 1.5-inch diameter RF coil was used in the experiment. Sample size ranges were typically 1.5~2.0 cm in diameter and 2.0 cm long. ^{1}H spin-spin (T_2) relaxation profiles were obtained by using the NMR CPMG (90° - τ - 180° -delay - echo - delay - 180° -) spin echo method with a 4.2 μs duration 90° pulse, τ of 500 μs, and a T_R (sequences repetition rate) of 10 s. For each T_2 profile, 800 echoes were acquired and 40 scans were used. The data were measured at room temperature after fresh frozen turkey ulnae (saturated with phosphate buffered saline, pH=7.4, a 99+% H_2O solution) were completely thawed at room temperature (21 ± 1°C).

Translation NMR data to pore size: In fluid saturated porous media, e.g., bone, T_2 values are shorter for pore fluids than for the bulk fluids since the fluids interact with the pore surface to shorten the NMR relaxation. In the fast diffusion limit, the T_2 relaxation rate $1/T_2$ is proportional to the surface-to-volume (S/V) ratio of the pore [6,7]:

$$1/T_2=\rho(S/V)_{pore} \qquad (1)$$

Where ρ is the surface relaxivity, which is a measure of the pore surface's ability to enhance the relaxation rate ($1/T_2$).

The T_2 spectral distribution can be correlated to the pore size distribution by using Equation (1) after knowing the relaxivity constant ρ. It is important to note that the relaxivity constant ρ should be determined by an in vitro experimental approach in bone samples. Generally and particularly in this study, we use bone

histomorphometry to provide the median pore size value and compare this to its corresponding T_2 inversion relaxation spectrum for its median T_2 relaxation time constant. Then, by using Eq. (1) ($1/T_2 = \rho (S/V)$), the ρ can be estimated. The relaxation spectrum was then analyzed for all samples, and pore size related to all samples was determined.

Validation of NMR measured pore size with histomorphometry determined porosity using SEM: After the NMR measurement, all samples were cut in half at mid-diaphyses. Bones were then embedded in polymethylmethacrylate. The cross section at mid-diaphyses was polished and silver coated. Each bone was then examined by a backscatter scanning electron microscopy (Model 1810D, AMRAY, Bedford, M.A.) at 8 equal pies with 50x and 120x magnification (Fig. 1). The images were then processed using custom-written software on PV-WAVE. The porosity for different channels, i.e., Haversian canals and lacunae was identified. The median pore size value was then used for estimating constant ρ and applying to calculate NMR determined porosity.

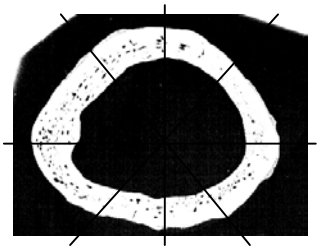

Figure 1. Histomorphometric image (SEM) of bone cross-section. The bone is divided into 8 equal pies for analysis.

RESULTS

NMR identified a variety of porosity in all tested bones, in which porosity ranged from 6.6% to 16.5% in averaged 8 pie sectors. NMR CPMG relaxation spectrum data have demonstrated significance for cortical bone samples from normal mature turkey ulna and disuse osteopenic bone (Fig. 2). The higher intensity signal was for the bone from disuse osteopenia with larger pore sizes, while the lower intensity signal was for the normal intact bone.

The distribution of porosity was validated by SEM histomorphometry, in which porosity ranged from 6.4% to 17.6%. A strong correlation between NMR determined porosity and SEM measured data was observed, R^2=0.98 (Fig. 3).

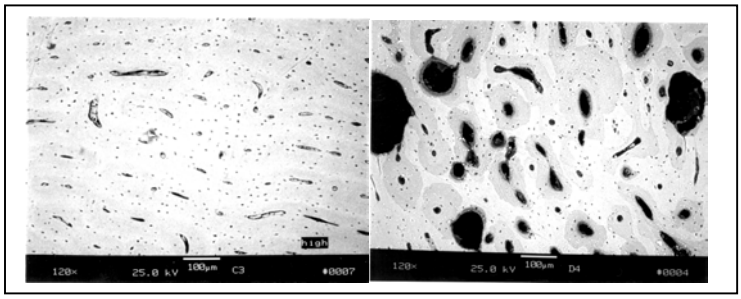

Figure 2. Backscatter electron microscope determined microstructure of bone porosity. Left – normal bone; right – osteoporotic bone after 4-week disuse.

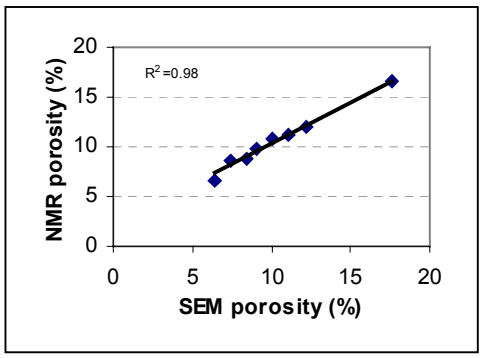

Figure 3. Correlation of between NMR determined and SEM measured porosities.

DISCUSSION

These data demonstrate that NMR predicted pore data are consistent with SEM measured bone porosity. These results imply that NMR, as a non-destructive method, can be used for predicting bone porosity in a relatively precise manner, if an appropriate verification procedure is applied, i.e., histomorphometric analysis such as SEM. As new modality for assessing bone porosity, NMR has capability for predicting pore size in micro scales, which represent the majority of bone fluid flow compartments, i.e., Haversian canals and lacuna-canaliculi.

There are several limitations for NMR predicted bone's porosity. (1) The NMR approach is sensitive enough to the soft tissues surrounding the bone samples because these water-saturated tissues may interfere the NMR signals induced by pores. Thus, carefully removal of soft tissue in the test will reduce the measurement errors. (2) The correlation data have shown that if SEM data approaches to 0% porosity, NMR shall show ~2% porosity remaining in the measurement. This inconsistency may be due to the accuracy of histomorphometric approach in assessing the true pore sizes in a 3-D scale. It is presumable that the NMR method may overestimate the true cortical porosity of bone, especially at low end of porosity, i.e., canaliculi. Multiple layers, 3-D, of SEM verification may help to reduce this inconsistency. Nevertheless, the relative accuracy of the NMR approach is in good agreement with the numerical prediction of bone fluid flow in this study. Histomorphometric analysis for more samples with multi-layer testing are necessary to translate NMR signals to bone porosity.

ACKNOWLEDGMENT

This work is kindly supported by The Whitaker Foundation and US Army Medical Research.

REFERENCES

1. Kelly, P. J., An, K.N., Chao, E.Y.S. and Rand, J.A., 1985, "Fracture healing: biomechanical, fluid dynamic and electrical considerations," Bone Min Res, Elsevier, New York. 295-319.
2. Piekarski, K. and Munro, M., 1977, "Transport mechanism operating between blood supply and osteocytes in long bones," Nature. 269:80-82,9-1-1.
3. Cowin, S.C., 1999, "Bone poroelasticity," J.Biomech. 32:217-238.
4. Weinbaum, S., Cowin, S.C. and Zeng, Y., 1994, "A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses," J.Biomech. 27:339-360.
5. Qin, Y. X., McLeod, K. and Rubin, C.T., 2000, "Intracortical fluid flow is induced by dynamic intramedullary pressure independent of matrix deformation," 46th Ann Mtg Orth Res Soc. 25:740-40.
6. Brownstein, K. R. and C. E. Tarr. Letter: Relaxation time versus water content: linear or nonlinear? Science. 194:213-214,10-8-1976.
7. Brownstein, K. R. and C. E. Tarr. Importance of Classical Diffusion in NMR Studies of Water in Biological Cells. Phys.Rev.A. 19:2446-1979.