REVIEW

Biophysic evaluation of bone quality -application of Fourier transform infrared spectroscopy and phosphorus-31 solid-state nuclear magnetic resonance spectroscopy-

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Abstract : In this review, we focus on findings obtained with biophysic techniques, Fourier transformed infrared (FTIR) spectroscopy and phosphorus-31 solid-state nuclear magnetic resonance (^{31}P solid-state NMR) spectroscopy, which may allow us to evaluate bone quality and to predict bone strength. FTIR measures the absorption energy that produces an increase in the vibrational or rotational energy of atoms or groups of atoms within the molecule. FTIR spectroscopy allows us to examine the relative amount of minerals and matrix content and the arrangement of apatite and organic matrix. FTIR spectroscopy should become an important tool, because the relative amount of minerals and the arrangement of apatite and organic matrix could be a measure for evaluating bone quality. ^{31}P solid-state NMR spectroscopy is useful for evaluating the quality of bone and predicting bone strength by calculating the spine-lattice relaxation time (T_1) of bone. ^{31}P solid-state NMR imaging can be used to measure quantitatively the mass of hydroxyapatite. The T_1 relaxation time of both bone and deficient hydroxyapatite was much longer than that of pure hydroxyapatite. T_1 relaxation time is one of the promising indices of bone quality. J. Med. Invest. 51 : 133-138, August, 2004

Keywords **:** *Fourier transform infrared spectroscopy, phosphorus-31 solid-state nuclear magnetic resonance spectroscopy, aging, bone quality, peripheral quantitative computed tomography*

INTRODUCTION

Bone mineral density (BMD) accounted for approximately 75-80% of the variance in bone strength (1). It is certain that BMD is one of the important determinants of bone strength. However, in our clinical experience, we have encountered some patients with high or normal BMD who had suffered an atraumatic bone fracture. On the other hand, some patients with low BMD have never had a bone fracture. Previous studies have shown that one of the determinants of bone strength, apart from BMD or bone mineral content, is the three-dimensional trabecular microstructure of bones (2-4). In order to predict bone strength and bone quality, we need new trustworthy parameters of bone quality. At present, a definition of bone quality has been difficult and controversial.

This review focused on the biophysic analysis of bone quality as a predictor of bone strength. We present two biophysic techniques, Fourier transformed infrared (FTIR) spectroscopy and phosphorus-31 solid-state

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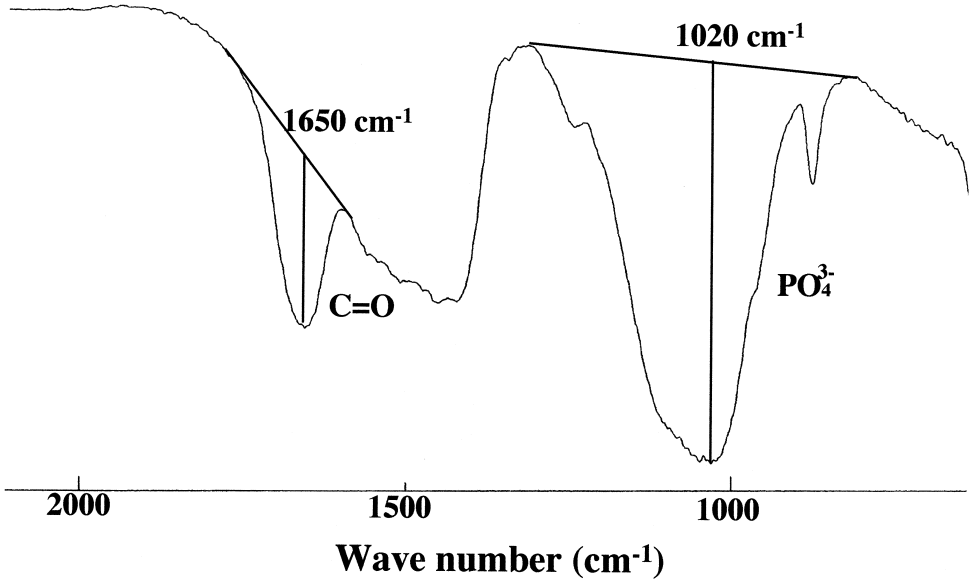

Fig.1 Typical spectra of the femoral shaft of rats obtained by FTIR. Peak positions near 1020 cm^{-1} and near 1650 cm^{-1} were assigned to the PO_4^{3-}-stretching vibrations of apatite and the C=O (amide 1)stretching vibrations of the bone organic matrix, respectively. The mineral / matrix ratio was calculated from the ratio of the absorbance of the phosphate band at 1020 cm^{-1} to that of the amide 1 band at 1650 cm^{-1}.

nuclear magnetic resonance (^{31}P solid-state NMR) spectroscopy, for evaluating bone quality. These techniques should become important in evaluating bone quality in the near future.

FOURIER TRANSFORM INFRARED (FTIR) SPECTROSCOPY AND FTIR IMAGING (FTIRI)

FTIR spectroscopy allows us to examine the molecular structure and conformation of biological macromolecules because it measures the absorption energy, which produces an increase in the vibrational or rotational energy of atoms or groups of atoms within the molecule (3, 5, 6). The information obtained from FTIR spectroscopy includes the relative amount of minerals and matrix content, the arrangement of apatite and organic matrix (7, 8), and carbonate to phosphate ratio (9). Paschalis *et al*. (7) used FTIR microspectroscopy to analyze single osteons in the iliac crest of normal humans, and showed that the mineral/matrix ratio increased from the center to the periphery of the osteon. If the relative amount of minerals and the arrangement of apatite and organic matrix are useful in evaluating bone quality, FTIR spectroscopy should become an important tool.

The spectra bands of interest in FTIR are both mineral bands and organic bands (Fig.1). The spectral resolution was 4 cm^{-1}. The abscissa range was 900-1800 cm^{-1}, covering the phosphate band and the amide

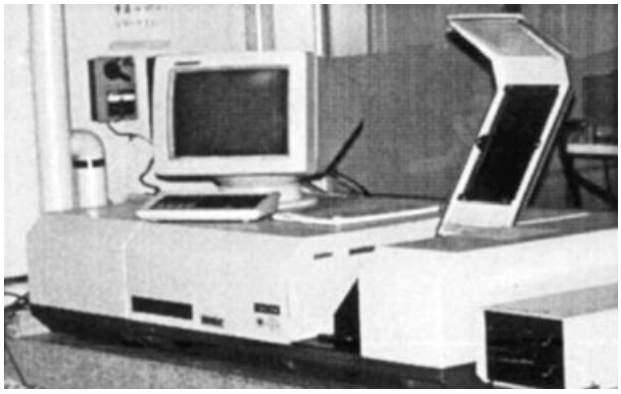

Fig.2 FTIR spectrometer (FTIR-1720, Perkin-Elmer, Norwalk, USA).

1 band. According to previous studies (5,10), peak positions near 1020 cm^{-1} and near 1650 cm^{-1} were assigned to the PO_4^{3-} stretching vibrations of apatite and the C=O (amide 1) stretching vibration of the bone organic matrix, respectively. Data were Fourier transformed and averaged after 50 scans. The mineral / matrix ratio was calculated from the ratio of absorbance of the phosphate band at 1020cm^{-1} to that of the amide 1 band at 1650 cm^{-1}.

Recently, we reported the mineral/matrix ratio of the rat femur, to clarify effects of disuse on bone (11). The infrared spectra were recorded by the FTIR spectrometer (FTIR-1720, Perkin-Elmer, Norwalk, USA) (Fig.2). The samples were examined by the KBr technique (6, 12). In our previous study (11), rat femurs were removed and soft tissue, periosteum and bone marrow were immediately cleaned off. The midshaft of the

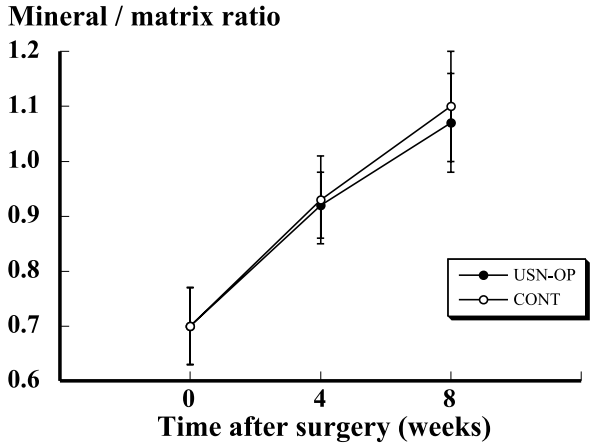

Fig.3 Time course of changes in mineral/matrix ratio of the femur in unilateral sciatic neurectomy-operated (USN-OP) and control (CONT) groups (11). The mineral/matrix ratio did not differ significantly between these two groups at 4 and 8 weeks after the surgery. The values are means±SD (n=5).

diaphysis was frozen in liquid nitrogen and lyophilized for 24 hours to remove all water, and then ground into liquid nitrogen. The mineral / matrix ratio increased 4 weeks and 8 weeks after the surgery, and we could not find significant changes in the mineral / matrix ratio of the rat femur between the disuse bone and control bone, indicating that bone quality as reflected by the mineral / matrix ratio in disused bone does not worsen (Fig.3).

Figure 4 shows the mineral / matrix ratio by FTIR and the bone mineral density by peripheral quantitative computed tomography of the femurs of male Wistar-derived albino rats aged 6 weeks and 36 weeks. The results show that the mineral / matrix ratio and bone mineral density of the femurs of rats ageds 36 weeks were significantly greater than those of rats aged 6 weeks ($p<0.0001$). This suggested that the mineral / matrix ratio assessed by FTIR spectroscopy becomes an important indicator of the maturation or aging of bone.

FTIR imaging (FTIRI) was used to investigate the therapeutic effects of estrogen on bone quality in early postmenopausal women, and showed definite changes in bone properties at the molecular level following hormone replacement therapy (13). Moreover, FTIRI analyses showed that recombinant human parathyroid hormone treatment decreased the mineral crystal maturity and collagen cross-link ratio (pyridinoline/dehydrodihydroxylysinonorleucine) on both periosteal and endosteal surfaces (14). Boskey *et al*. (15) showed an increased collagen maturity in both the cortical and trabecular bone of the osteonectin-null mice using FTIRI.

^{31}P SOLID-STATE NMR SPECTROSCOPY AND IMAGING

^{31}P solid-state NMR spectroscopy using magic angle spinning has been used to study the chemical structure of bone mineral (6, 16-18). ^{31}P solid-state NMR spectra were obtained on a custom-built pulse spectrometer operated at 119.1 and 128.6 MHz for phosphorus (19). Powdered samples of bone were tightly packed into a ceramic double-bearing rotor. The sample spinning rates were 2.0 KHz and 2.16 KHz for facilitating comparisons of line intensities among samples

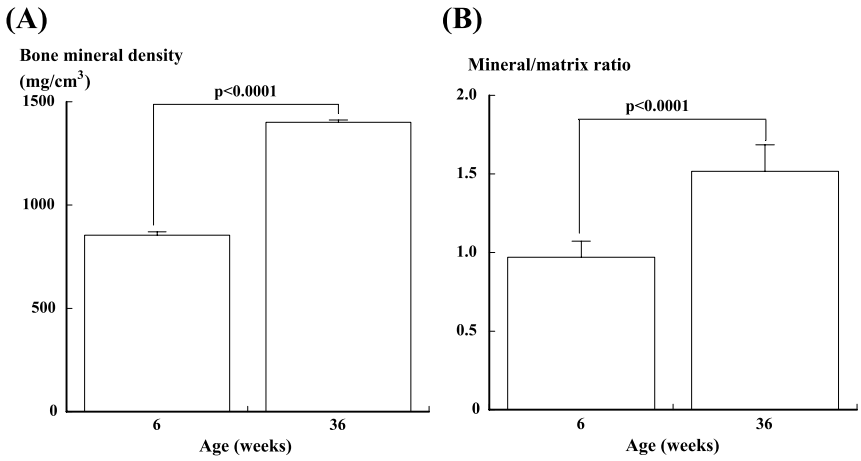

Fig.4. Bone mineral density (A) and mineral/matrix ratio (B) of rat femurs aged 6 weeks and 36 weeks. The bone mineral density of femurs aged 36 weeks was significantly higher than that of femurs aged 6 weeks ($p<0.0001$). The mineral/matrix ratio of femurs aged 36 weeks was significantly greater than that of femurs aged 6 weeks ($p<0.0001$). The values are means±SD (n=5). Student's unpaired t test was used to evaluate differences between these two groups.

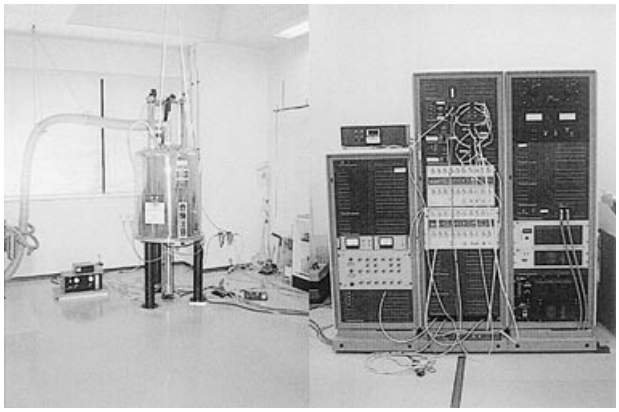

Fig.5 An 8.5-Tesla superconducting spectrometer (CMX-960, CHEMAGNETICS, s INC., Colorado).

and between spectrometers.

^{31}P solid-state NMR imaging can be used to measure quantitatively the mass of hydroxyapatite, a synthetic calcium phosphate used as an orthopedic implant material. This technique can be used to follow noninvasively the resorption and remodeling of calcium phosphate implants *in vivo* (20). Marchandise *et al*. (21) used magic angle sample spinning ^{31}P solid-state NMR spectroscopy to study the NMR parameters of hydroxyapatite, calcium-deficient hydroxyapatite and beta-tricalcium phosphate. The results showed that the spectrum of rabbit bone is similar to that of deficient hydroxyapatite, and that the T_1 relaxation times of both bone and deficient hydroxyapatite were much longer than that of pure hydroxyapatite.

Dawson *et al*. (22) applied ^{31}P solid-state NMR spectroscopy to characterize and quantitate bone mineral and a synthetic apatite, to establish a model for bioabsorption studies, and suggested that ^{31}P solid-state NMR is a suitable technique for the *in vitro* analysis of bone specimens. ^{31}P solid-state NMR spectroscopy allows us to examine a number of synthetic crystalline and noncrystalline $CaPO_4$. Aue *et al*.(23) showed that isotropic and anisotropic chemical shifts together with proton-suppression techniques can be used to differentiate synthetic $CaPO_4$ compounds from one another. This technique permitted us to exclude certain $CaPO_4$ solid phases as major or minor phases in bone, but it has also made it possible to define much more clearly the nature of the mineral phases in bone.

In our recent study, an 8.5-Tesla superconducting spectrometer (CMX-960, CHEMAGNETICS, INC., Colorado) was used to obtain ^{31}P solid-state NMR spectra (Fig.5). Casella *et al*. showed that the chemical shift of phosphorus of normal human bone is 3.3 ± 0.1 ppm (6). Figure 6 shows a typical spectrum of the femoral shaft of male Wistar-derived albino rats obtained by ^{31}P solid-state NMR spectroscopy in our recent study. The chemical shift of phosphorus of the rat femur is approximately 3.3ppm. We measured the T_1 relaxation time of male rat femurs aged 6 weeks and 36 weeks to study the effects of aging and development on T_1 relaxation time and bone strength (Figs.7 A, B). The T_1 relaxation time and bone strength of the male rat femurs aged 36 weeks were significantly greater than those aged 6 weeks ($p<0.0001$). These results suggest that T_1 relaxation time is one of the promising indices

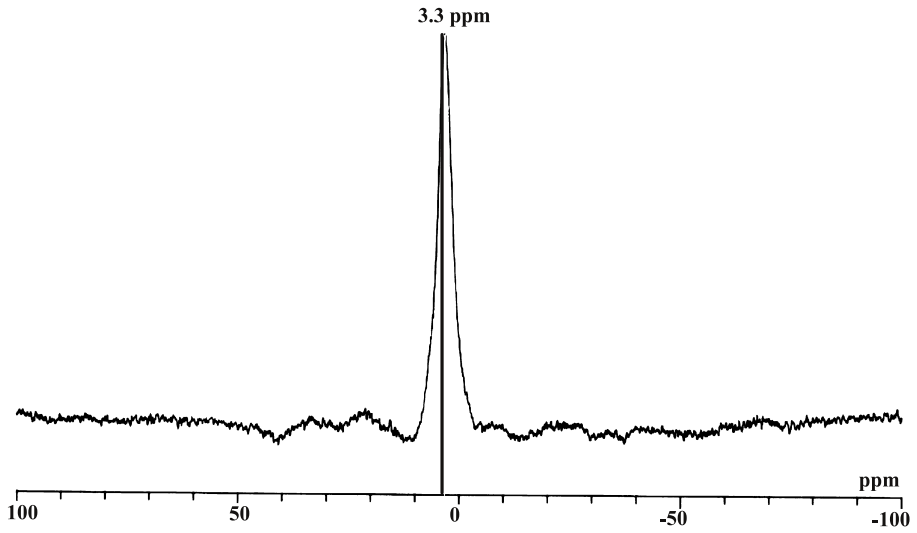

Fig.6 A typical spectrum of the rat femoral shaft of obtained by ^{31}P solid-state NMR. An 8.5-Tesla superconducting spectrometer was used to obtain this spectrum. The chemical shift of phosphorus is 3.3 ppm.

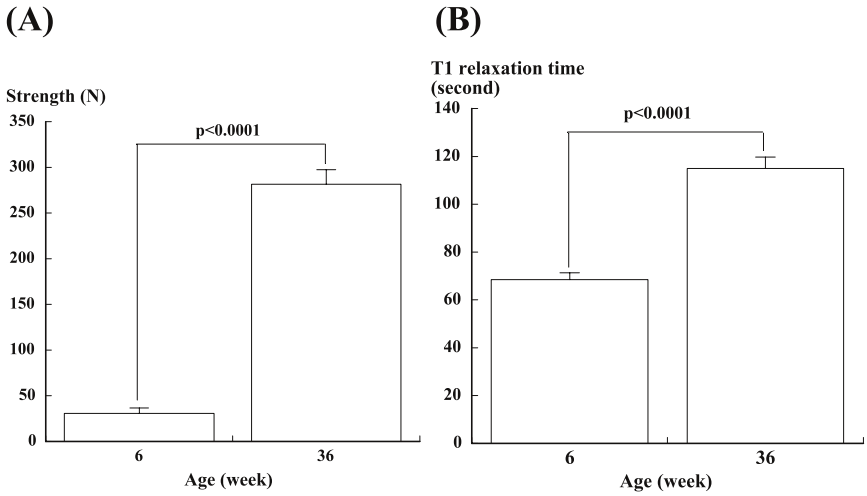

Fig.7 Bone strength (A) and T_1 relaxation time (B) of male rat femurs. The bone strength of femurs aged36weeks was significantly greater than that of rat aged6weeks ($p<0.0001$). T_1 relaxation time of femurs aged 36 weeks was significantly greater than that of femurs aged 6 weeks ($p<0.0001$). The values are means±SD (n=5). Student's unpaired t test was used to evaluate differences between these two groups.

of bone strength and bone quality. ^{31}P solid-state NMR spectroscopy is a promising technique for evaluating the quality of bone and predicting bone strength by calculating the T_1 relaxation time of bone.

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