REVIEW

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

Shinjiro Takata1), Akira Shibata2), Hiroshi Yonezu3), Toshihide Yamada4), Mitsuhiro Takahashi5), Aziz Abbaspour6), and Natsuo Yasui7)

1)Department of Orthopedics, and 2)Faculty of Pharmaceutical Sciences, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan; 3)Department of Orthopedic Surgery, Shikoku Chuo Hospital, Ehime Japan; and 4)Formulation Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan

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 (31P 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. 31P solid-state NMR spectroscopy is useful for evaluating the quality of bone and predicting bone strength by calculating the spine-lattice relaxation time (T1) of bone. 31P solid-state NMR imaging can be used to measure quantitatively the mass of hydroxyapatite. The T1 relaxation time of both bone and deficient hydroxyapatite was much longer than that of pure hydroxyapatite. T1 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 trabecular microstructure 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
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 1 band. According to previous studies (5,10), peak positions near 1020 cm$^{-1}$ and near 1650 cm$^{-1}$ were assigned to the PO$_4^-$ stretching vibrations of apatite and the C=O (amide I) 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$^{-1}$.

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
The mineral density of the femurs of rats aged 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/dehydroxylysinosinorleucine) 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 osteonecrosis-null mice using FTIRI.

\[ ^{31}\text{P} \text{SOLID-STATE NMR SPECTROSCOPY AND IMAGING} \]

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

---

**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).

**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.
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 non-invasively 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 CaPO₄. Aue et al. (23) showed that isotropic and anisotropic chemical shifts together with proton-suppression techniques can be used to differentiate synthetic CaPO₄ compounds from one another. This technique permitted us to exclude certain CaPO₄ 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 $3.3 \pm 0.1$ 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.5](image1.png)

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

![Fig.6](image2.png)

**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.
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.

REFERENCES

12. Weiss P, Bohic S, Lapkowske M, Daculsi G:


S. Takata et al.  Biophysical evaluation of bone quality