

# Chemical Structure Effects on Bone Response to Mechanical Loading

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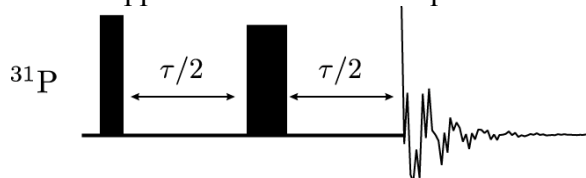
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## INTRODUCTION

Solid state nuclear magnetic resonance (SSNMR) spectroscopy is a powerful tool for studying high-resolution structural changes in a wide variety of materials, including materials subjected to high pressure (Redfern, 2000). We show that SSNMR can probe load-induced changes in the chemical structure of bone mineral and bone matrix. We use magic angle spinning NMR spectroscopy (MAS-NMR) and a specially designed load cell, to probe changes in the ion spacings of bovine cortical bone mineral in response to compressive loads in the MPa region.

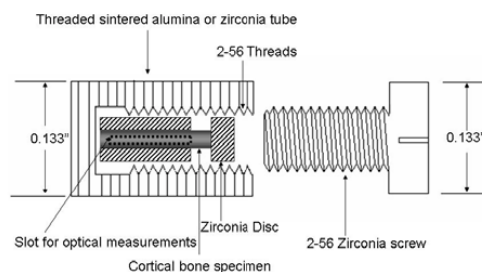
## METHODS AND PROCEDURES

For these studies, <sup>31</sup>P NMR of bone mineral phosphate was investigated. All NMR experiments were performed on a Chemagnetics/Varian 400MHz solid-state NMR spectrometer equipped with a 5mm double tuned MAS probe. The spin-echo pulse sequence outlined in figure 1 was used. A purpose-built cell was used to apply known loads to the tissue specimens. The cell was constructed entirely from ceramics. Loads were applied with a torque wrench.



**Figure 1.** Spin-echo pulse sequence to measure <sup>31</sup>P isotropic chemical shifts from bone specimens

under MAS. The first and second solid blocks are 90 and 180 degree RF pulses.



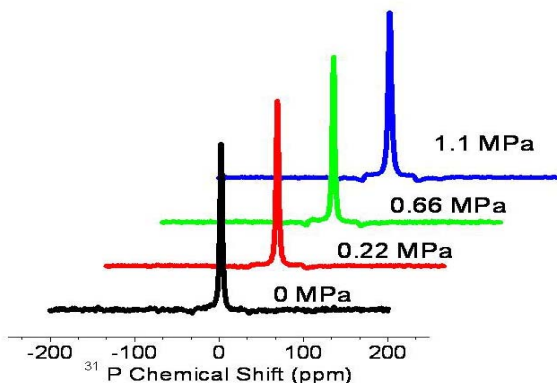
**Figure 2.** NMR cell to apply a high pressure load with a torque wrench. The cell fits into a standard MAS rotor. The slot is for optical measurements of strain.

## RESULTS

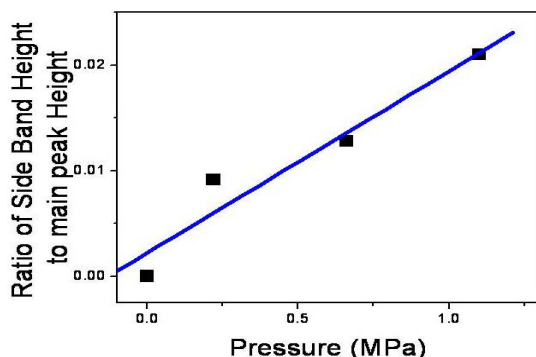
In our first proof of principle experiments we were able to load the tissue only to about 1.1 MPa. The limitation was failure of the ceramic NMR cell. Our initial tests of new ceramics confirm that we can reach about 4 MPa with no other changes. Further changes in the cell design should allow us to reach loads in the 8-10 MPa range.

We observed that the resonance frequency of phosphate was independent of load (Figure 3). Our earlier experiments were performed at a low spinning frequency, resulting in the spinning side bands shown in the figure. The intensity of spinning side bands increases linearly with the load (Figure 4). The reason for this observation is that homonuclear and heteronuclear dipolar coupling constants, which measure the interaction between

adjacent atoms or ions, increase linearly with the inverse cube of spacing, i.e. with the inverse of volume. The scaling is the same for pressure-induced volume changes. Thus, the slope of the curve is directly proportional to the decrease in mineral volume accompanying the load. The same linear change with pressure is expected for any effect that depends linearly on the dipolar coupling.



**Figure 3.**  $^{31}\text{P}$  NMR spectra of bone mineral as a function of compressive load. Applied pressures are included. Intensity of spinning side bands increase with pressure.



**Figure 4.** Spinning side band intensity of bone mineral  $^{31}\text{P}$  resonance scales linearly ( $R^2=0.97$ ) with the compressive load.

## DISCUSSION

No observed changes in the isotropic and anisotropic chemical shift values of  $^{31}\text{P}$  nuclei under the compressive load suggest that the

chemical environment of the phosphate group in the bone sample is unaltered. However, bone samples under pressure showed a significant increase in the intensity of spinning sidebands, suggesting that dipolar couplings are not completely suppressed by the MAS speed. This observation is most likely due to the pressure-induced decrease in the distance between dipolar coupled nuclei. As with most minerals, loads in the MPa range changes ion spacings, but does not deform covalent bonds.

Most importantly, these results demonstrate that a wide variety of biomechanical NMR studies of bone are possible. In particular, any effect that results in a linear dependence of dipolar coupling constants will be amenable to relatively straightforward interpretation.

## SUMMARY

Solid-state MAS-NMR can be used to measure atomistic-level changes in the chemical structure of bone tissue caused by mechanical load. Where NMR techniques that use dipolar coupling are employed, linear dependences on pressure are expected and spacing between ions in matrix or between nuclei in bone collagen can be measured directly.

## REFERENCES

Redfern SAT et al (2000). *Transformation Processes in Minerals (Reviews in Mineralogy and Geochemistry)*, Vol 39 .

## ACKNOWLEDGEMENTS

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