

# BONE CHEMICAL STRUCTURE RESPONSE TO MECHANICAL STRESS STUDIED BY HIGH PRESSURE RAMAN SPECTROSCOPY

Olivia de CarneJane<sup>1</sup>, Michael D. Morris<sup>1</sup>, Rupak M. Rajachar<sup>2,3</sup>, M. Kathleen Davis<sup>4</sup>, Lars Stixrude<sup>4</sup>, Mary Tecklenburg<sup>5</sup>, David H. Kohn<sup>2,3</sup>

<sup>1</sup>Chemistry, <sup>2</sup>Biologic and Material Sciences, <sup>3</sup>Biomedical Engineering, <sup>4</sup>Geological Sciences, University of Michigan, Ann Arbor, MI, 48104

<sup>5</sup>Chemistry, Central Michigan University, Mt. Pleasant, MI 48859

E-Mail: [dhkohn@umich.edu](mailto:dhkohn@umich.edu)

## INTRODUCTION

Age related skeletal fragility is a significant medical problem involving up to 250,000 fractures/year. Bone mass is not always an effective predictor of fragility, implying that measures of bone quality, such as composition, are also important variables.

We have reported Raman spectra showing the presence of both uncarbonated apatitic mineral and highly disordered carbonated mineral at the leading edge of fatigue-induced microcracks (Timlin et al., 2000) and at sites of catastrophic fractures in cortical bone subjected to bending (Morris et al., 2002). These studies, however, did not resolve whether the altered mineral phases were a cause or result of damage. Indentation/Raman spectroscopic imaging studies confirmed our hypothesis that changes in mineral composition result from deformation (Carden et al., 2003).

We now further hypothesize that phase changes in the mineral lattice of bone are pressure-dependent and occur concurrently with matrix changes. To address this hypothesis we assess Raman spectroscopic response of murine cortical tissue to hydrostatic pressure. These studies further demonstrate cause/effect relations between mechanical loading and compositional changes via collection of Raman data in real-time as a function of mechanical load.

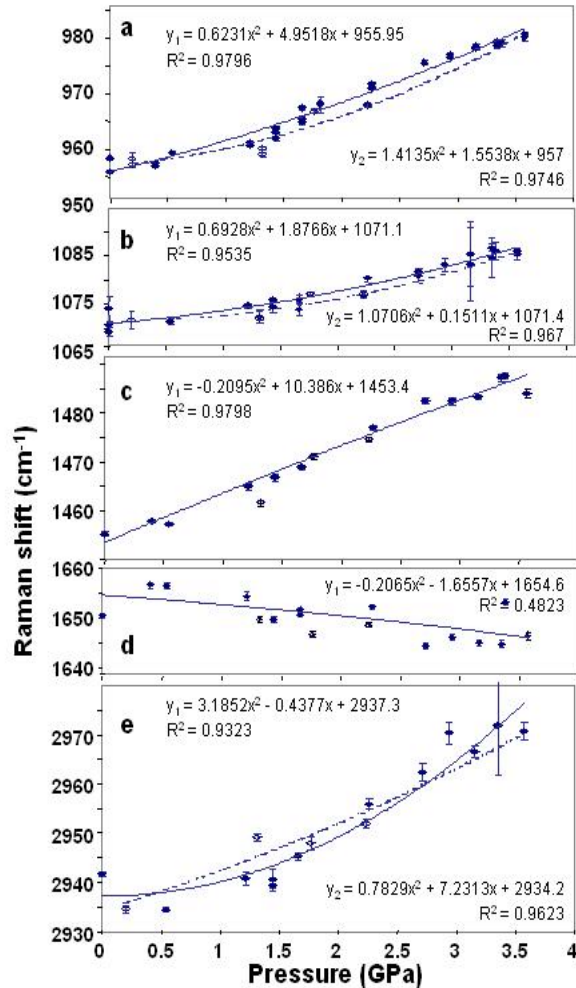
## METHODS

**Specimens.** Murine femora were harvested, stripped of soft tissue, wrapped in gauze soaked in Ca-buffered saline and stored at -30°C prior to handling. Both bone and deproteinated bone were analyzed. Bones were pulverized by snap freezing in liquid N<sub>2</sub> followed by grinding with mortar and pestle. Another set of bones was deproteinated in hydrazine with washing in ethanol. Powders were stored in Ca-buffered saline at 4°C (deproteinated bone) or -30°C (bone powder).

**High Pressure Raman Spectroscopy.** High pressure experiments (up to 3.8 GPa) employed a Bassett-type diamond-anvil cell with rhenium gaskets. The chamber was loaded with selected bone crystallites and a drop of deionized water (or deuterated water) was added as coupling fluid. At each pressure increment the hydrostatic pressure inside the cell was monitored as the 2.9 cm<sup>-1</sup>/GPa shift of the diamond Raman band at 1332 cm<sup>-1</sup>. In situ Raman spectra were collected in the 100-3800 cm<sup>-1</sup> frequency range. Measurements were made under loading and unloading. Raman spectra were excited at 514.5 nm with Ar<sup>+</sup> laser operating at 2 W focused to a 2 μm spot through an epi-illumination microscope with a 50X objective. The scattered radiation was analyzed with a spectrograph operated at 2 cm<sup>-1</sup> resolution and a cooled CCD detector.

**Data Analysis.** Spectra were baselined and bands were fitted to mixed Gaussian-Lorentzian line shapes to define band positions. Regression analyses were applied to mineral and matrix bands.

## RESULTS AND DISCUSSION



**Figure 1:** Pressure dependence of A)  $\text{PO}_4^{3-} \nu_1$ , B)  $\text{CO}_3^{2-} \nu_1$ , C)  $\text{CH}_2$  wag, D) Amide I and E)  $\text{CH}_2$  stretch Raman shifts for mouse bone. Loading: closed symbols and quadratic fit  $y_1$ ; unloading: open symbols and quadratic fit  $y_2$ .

Shifts to higher wavenumbers were observed for most of the bands as pressure increased (Fig 1). Regression analyses determined that the pressure-dependence was a second-order relation. Since carbonate

is a small, hard ion that cannot be deformed, we propose that the pressure dependence (Fig 1B) is due to movement of oxy-anions and cations within the apatitic unit cell. Amide I (Fig 1D) shows a small negative pressure dependence. The weak pressure dependence is surprising, because this band responds to changes in secondary structure and hydrogen bonding.

Unloading resulted in a return to the original band position. Comparison of band envelopes at atmospheric pressure before and after loading revealed no permanent changes such as band broadening or shoulders, implying that either the changes are reversible or the fraction of conversion is too small to measure in this experiment.

## SUMMARY

Bone responds actively to mechanical stress at the lattice-level in a pressure-dependent fashion. This ultrastructural-level response is more complex than has been recognized. The spectral shifts are consistent with changes in unit cell parameter with little or no change in oxy-anion X-O bond lengths. The large changes in methylene wag are consistent with either changes in the pitch of the collagen helix or with changes in interfibril links.

## REFERENCES

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