A novel assessment of bone quality using time-resolved transcutaneous Raman spectroscopy

E R C Draper, A E Goodship

Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire AL9 7TA and Institute of Orthopaedics and Musculoskeletal Sciences, University College London, Stanmore, Middlesex, HA7 4LP, UK

M D Morris

Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, USA

N P Camacho

Hospital for Special Surgery, 535 East 70th Street, New York, NY 10021, USA

P Matousek, M Towrie, A W Parker

Central Laser Facility, CCLRC Rutherford Appleton Laboratory, Chilton, Didcot, Oxon., OX11 0QX, UK

Main contact email address: *e.draper@imperial.ac.uk*

Introduction

The major clinical demand for bone quality assessment arises from the devastating disease, osteoporosis, which leads to 1.5 million fractures per annum in the USA[1]. With forecasts of a more aged population we can expect this to get worse: fragility fractures of the hip, for instance, are expected to rise worldwide from 1.3 million in 1990 to 2.3 million in 2020[2]. Preventative strategies, as well as treatment of fractures once they have occurred[2], will be needed to meet this escalating problem.

Dual-Energy X-ray Absorptiometry (DXA), although the current gold standard screening modality, is able only to account for 60 to 70% of the variation in bone strength[3]. It is clear that BMD is not the sole predictor of fracture risk[4,5] important factors are being missed. Both infrared and Raman spectroscopies have been explored as a means of assessing bone tissue to provide this information; they have been used to measure mineral/matrix ratio (related to bone material density), mineral crystallinity (related to mineral quality) and crosslinking within the organic phase (related to collagen quality)[6-8]. To date these techniques have been applied directly to specific excised tissue samples. Until this present study, it had not been possible to perform vibrational spectroscopy of healthy bone at useful depths through overlying tissue. We overcame these shortcomings by using a picosecond pulsed laser at a wavelength with good tissue penetration (800 nm) with an ultrafast, picosecond shutter (Kerr-gate[9,10]).

The aim of this study, therefore, was to test the hypothesis that spectral features of both the mineral and organic phases of bone specimens with known differences in material properties can be measured objectively through the unbroken skin using time-resolved Raman spectroscopy.

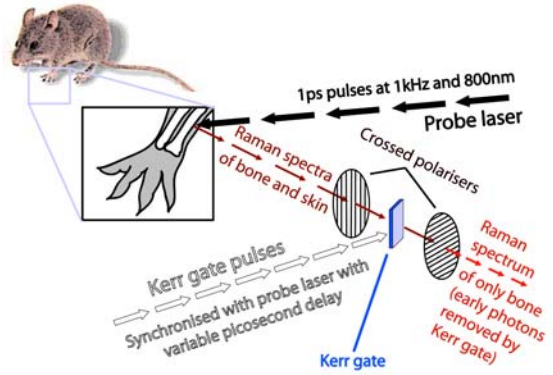

Figure 1. Cartoon of experimental set up.

Materials and Methods

Laser setup

The description of the system we used for Kerr-gated picosecond Raman spectroscopy has been described previously[9]. In brief, a femtosecond mode-locked Ti:sapphire laser was used to produce a 1 kHz pulse train, which was then amplified by a Nd:YLF-pumped regenerative amplifier to produce 1 ps, 800 nm pulses at 2 mJ at 1 kHz. A 500 µJ pulse was isolated from this by a beam splitter to drive a 4 ps Kerr shutter; the remainder of the pulse was frequency-doubled and used to drive an optical parametric amplifier. The time interval between the two pulses was controlled with an optical delay line to an accuracy of 50 fs with a delay up to 4 ns; our experiments used delays between 0 to 20 picoseconds. The pulse energy at

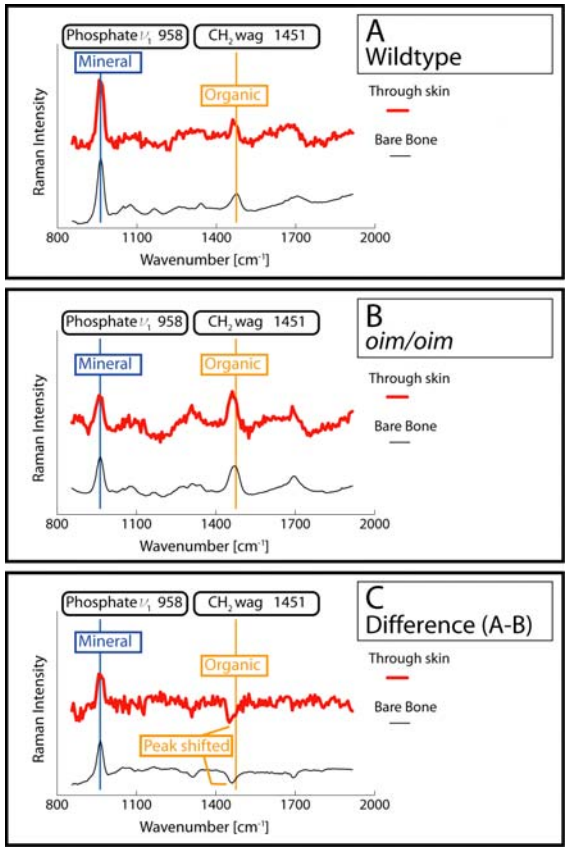

Figure 2. Raman spectra of bone measured from two genotypes; wildtype mice and oim/oim mice. A wildtype mice B oim/oim mice C difference Raman spectra.

the specimen at this wavelength was 8 μJ and the average power was 8 mW focussed to a spot size of 1 mm. We employed a conventional Raman spectrograph (Spex, Horiba Jobin Yvon Inc.) and back-illuminated deep depletion CCD (Andor Technology, DU420BR-DD) with the probe laser at an angle of 180º to the collection path (backscattering geometry). The overview of this setup and the principle by which it is possible to measure Raman spectra through overlying structures is summarised in the cartoon in Figure 1.

Mouse samples

Two genotypes, B6C3Fe Col1a2oim/Col1a2oim mice, commonly known as oim/oim and their wildtype controls were utilized. The oim mutation produces an alteration in type I collagen that affects not only the collagen but also the level of mineralisation and results in a weaker bone[11]. We used forelimbs from three skeletally mature mice from each genotype. The mice were matched for age, sex and weight. Each limb was shaved and mounted in a purpose-built jig with cranial surface facing the probe laser. Heating of the specimens by the laser was prevented by slow water irrigation. The site probed by the laser probe site we chose was 2 mm above the carpus. As a control, following transcutaneous measurements, the overlying soft tissues were carefully excised down to bare bone, the exposed bone then re-measured using the same irrigation both at the same site and at a point at the midpoint of the humerus.

Results

Typical Raman spectra from bone are well established[6], with different specific regions being associated with the mineral and the organic phases. We have studied two features: i) the phosphate ν1 band of the apatite of the mineral phase and ii) the band associated with the CH_2 wag of the collagen of the organic phase, Figure 2.

It can be seen from the spectra recorded from the exposed bone that there are considerable differences between the two genotypes, Figures 2A and 2B. The difference spectra in Figure 2C clearly indicate what is altered between the genotypes. Not only is it obvious that the heights of some of the spectral bands have changed but also, interestingly, the wavenumber of the band of the CH_2 wag has shifted.

By examination of the ratios of the band areas associated with phosphate ν1 and the CH_2 wag between the two genotypes, we can see a significant difference of the mean ratio between the genotypes both measured through the skin (p=0.007) and directly from the bone (p=0.005), Figure 3. Furthermore, there is no significant difference in mean area ratio for the same genotype whether measured through skin or from bare bone (p>0.05).

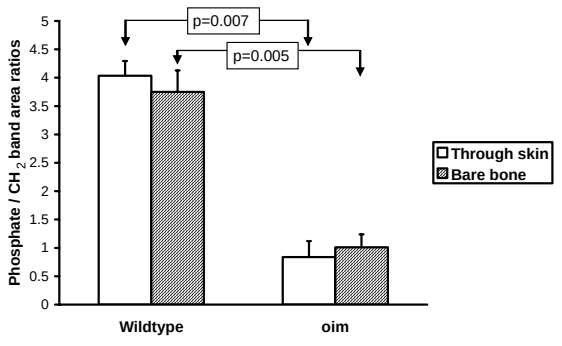

Figure 3. Ratio band areas phosphate ν1 band and CH_2 wag of organic phase for each genotype (mean and coefficient of variation).

Discussion

Our study clearly shows that major components of the Raman spectrum of bone can be detected without excision of bone or even cutting the skin. Major bands associated both with the mineral and with the organic phases[6] are clearly visible in the spectra measured through skin in Figure 2. Interestingly, our technique through the skin was sufficiently sensitive to record a slight variation of the wavenumber of the peak associated with collagen (the CH_2 wag at ~1451cm^{-1}) between the genotypes, Figure 2C.

We conclude, therefore, that the time-gated Raman spectroscopic technique has allowed for the first time vibrational spectral measurements of both the mineral and organic phases of bone tissue through the unbroken skin. We are embarked on the optimisation of the instrumentation to detect more subtle differences and be more specific as to the spectral features of importance. If we are successful, we anticipate that this technology will help improve fracture risk assessments probably in conjunction with other measurements such as DXA.

References

1. J Placide, M G Martens,
Comparing screening methods for osteoporosis. Curr Womens Health Rep 3:207-210, (2003).

2. L J Melton, O Johnell, E Lau, C A Mautalen, E Seeman,
Osteoporosis and the global competition for health care resources. J Bone Miner Res;19:1055-1058, (2004).

3. P Ammann, R Rizzoli,
Bone strength and its determinants. Osteoporos Int;14 Suppl 3:S13-S18, (2003).

4. T M Boyce, R D Bloebaum,
Cortical aging differences and fracture implications for the human femoral neck. Bone;14:769-778, (1993).

5. D Marshall, O Johnell, H Wedel,
Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. BMJ;312:1254-1259, (1996).

6. A Carden, M D Morris,
Application of vibrational spectroscopy to the study of mineralized tissues, J Biomed Opt;5:259-268, (2000).

7. C P Tarnowski, M A Ignelzi Jr., M D Morris,
Mineralization of developing mouse calvaria as revealed by Raman microspectroscopy. *J Bone Miner Res*;17:1118-1126, (2002).

8. C P Tarnowski, M A Ignelzi Jr, W Wang, J M Taboas, S A Goldstein, M D Morris, Earliest mineral and matrix changes in force-induced musculoskeletal disease as revealed by Raman microspectroscopic imaging. J Bone Miner Res;19:64-71, (2004).

9. P Matousek, M Towrie, A Stanley, A W Parker,
Efficient rejection of fluorescence from Raman spectra using picosecond Kerr gating. Applied Spectroscopy;53:1485-1489, (1999).

10. P Matousek, M Towrie, C Ma, W M Kwok, D Phillips, W T Toner, A W Parker,
Fluorescence suppression in resonance Raman spectroscopy using a high-performance picosecond Kerr gate. *Journal of Raman Spectroscopy*;32:983-988, (2001).

11. C A Miles, T J Sims, N P Camacho, A J Bailey,
The role of the alpha2 chain in the stabilization of the collagen type I heterotrimer: a study of the type I homotrimer in oim mouse tissues. *J Mol Biol*;321:797-805, (2002).