

CLINICAL CASES

Measurement of abnormal bone composition *in vivo* using noninvasive Raman spectroscopyKevin Buckley^{1,2}, Jemma G Kerns², Panagiotis D Gikas^{2,3}, Helen L Birch², Jacqueline Vinton³, Richard Keen³, Anthony W Parker^{1,2}, Pavel Matousek^{1,2} and Allen E Goodship²¹Central Laser Facility, Research Complex at Harwell, STFC Rutherford Appleton Laboratory, Harwell Oxford, UK. ²UCL Institute of Orthopaedics and Musculoskeletal Science, London, UK. ³Royal National Orthopaedic Hospital, Stanmore, UK.

X-ray-based diagnostic techniques, which are by far the most widely used for diagnosing bone disorders and diseases, are largely blind to the protein component of bone. Bone proteins are important because they determine certain mechanical properties of bone and changes in the proteins have been associated with a number of bone diseases. Spatially Offset Raman Spectroscopy (SORS) is a chemically specific analytical technique that can be used to retrieve information noninvasively from both the mineral and protein phases of the bone material *in vivo*. Here we demonstrate that SORS can be used to detect a known compositional abnormality in the bones of a patient suffering from the genetic bone disorder, osteogenesis imperfecta, a condition which affects collagen. The confirmation of the principle that bone diseases in living patients can be detected noninvasively using SORS points the way to larger studies that focus on osteoporosis and other chronic debilitating bone diseases with large socioeconomic burdens.

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Introduction

Musculoskeletal diseases have a large and growing socio-economic cost in countries with ageing populations; for example, in the UK there are over 70 000 hip fractures associated with osteoporosis and over 70 000 primary hip replacements associated with osteoarthritis annually.^{1,2} Presently, the range of clinical tools for early detection of these diseases is limited. In the case of osteoporosis, populations at higher risk of a first fragility fracture are identified using an association with bone density; however, the sensitivity of this method for an individual patient is <40%.³ Osteoarthritic patients usually seek treatment when they feel pain, at which point much of the affected joint can already be damaged or destroyed. The development of new screening and/or diagnostic tools for musculoskeletal diseases with higher sensitivity would enable earlier detection and treatments and could lead to a reduction in serious and costly clinical events. In the present study, we assess Spatially Offset Raman Spectroscopy (SORS), a variant of Raman spectroscopy that allows the retrieval of rich chemical information from a target layer through a turbid medium,^{4,5} as a bone-disease detection tool.

Osteogenesis imperfecta (OI) is a class of genetic disorders in which mutations in the collagen genes, or mutations in the genes associated with collagen (for example, those associated

with collagen post-translational modifications), affect the performance of the skeleton. As there are hundreds of mutations associated with OI, there are hundreds of phenotypic manifestations.^{6,7} Some of these mutations affect the skeleton so badly that they result in death *in utero* or shortly after birth (corresponding to type II in the Sillence classification of OI). Other mutations severely affect the skeleton and result in multiple fractures and short stature (roughly corresponding to type III and type IV in the same classification system). The mildest phenotypic manifestations (roughly corresponding to type I) can weaken the bones but in some cases are difficult to diagnose using X-ray-based techniques.

Patient Characteristics

The patient was a 26-year-old female with type IV OI. She had previously received treatment with Risedronate for 14 years, although this treatment was discontinued in November 2011 following a fracture to the femur. The patient underwent a wedge osteotomy in order to straighten her femur in February 2012 at the Royal National Orthopaedic Hospital and donated (with appropriate consent and ethical approvals) a section of bone that was ~0.5 cm thick at one end and tapered to 0.1 mm. It was cut from the mid-shaft of the femur.

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Determining the compositional abnormality of the patient's bone

The section of excised OI bone was probed with Raman spectroscopy and compared with non-OI bone that was excised (with appropriate ethical approvals) from the cadaveric tibiae of another female (aged 72 years). The Raman data were collected from the two excised sections with an inVia Raman microscope (Renishaw plc., Gloucestershire, UK) equipped with an 830 nm 200 mW laser (~ 10 mW at the sample). Twelve 1 min (60×1 s) spectra were collected from each excised sample and averaged. It was found that the OI patient's bone was significantly more mineralised compared with non-OI control bone; specifically, the phosphate ν_1 to Amide III ratio was $\sim 11\%$ higher (**Figure 1a**). The probing with Raman spectroscopy did not require any sample preparation; the laser was simply directed onto fresh bone samples.

Transcutaneous *in vivo* measurement

The OI patient returned to the hospital 10 months after her femur operation and her bones were scanned, transcutaneously, with a custom-built SORS instrument which utilised an 830 nm, 300 mW laser (Cobalt Light Systems, Oxfordshire, UK). The data were collected using the inverse SORS geometry—that is, an annular laser beam with the Raman collection point at the centre of the ring.⁸ The laser power in the ring was capped at 30 mW per 3.5 mm diameter aperture (as per BS EN 60825-1:2007, the safety standard relevant to laser light on the skin). The power automatically increased with increasing illumination area but stayed within the safety limit (BS EN 60825-1:2007). Spectra were collected from phalanges in the hands (five 0 mm offset, one 2.5 mm offset and six 5 mm offset spectra) and from the tibiae (one 0 mm offset and three 8 mm offset, collected at the midpoint of the bone). The accumulation time for each spectrum was 60 s (60×1 s); hence, in total 16 min data were

collected. The OI patient's DEXA Z-scores at the time of the SORS scans were -1.7 , -2.5 and -2.3 at her left hip, spine and right forearm, respectively. Control (non-OI) data were collected from a 20.5-year-old female volunteer using the same protocol.

Processing of transcutaneous *in vivo* data

The 32 Raman spectra were corrected for the charge-coupled detector's varying sensitivity across the spectra range and the broad fluorescence was removed from each spectrum by fitting a fourth order polynomial from 735 to 1540 cm^{-1} .⁹ The spectra were then normalised to the lipid band at ~ 1303 cm^{-1} .

In order to remove the surface signals (that is, from the skin and lipid layers), the spectra were decomposed using Band Target Entropy Minimisation (BTEM).^{10,11} The BTEM decomposition was repeated 15 times for the patient and 15 times for the control (that is, the whole-data set, with one spectrum left out in turn, was analysed for each individual); the algorithm was applied to sections of each spectrum (735 – 1384 cm^{-1}) and to the dominant phosphate band (955 – 970 cm^{-1}); fifteen eigenvectors were used. The 15 resultant spectra were averaged and s.d. calculated.

The SORS spectra show that, as expected from the excised measurements, the bone of the OI patient is more mineralised compared with the bone from the age-matched control; specifically, the mineral-to-protein ratio (phosphate ν_1 to amide III ratio) of the OI patient's bone is $\sim 14\%$ higher than that of the control bone (**Figure 1b**).

Discussion

SORS has been used to detect bone compositional abnormalities in an OI patient whose bones are known to have an increased ratio of mineral to protein (from excised

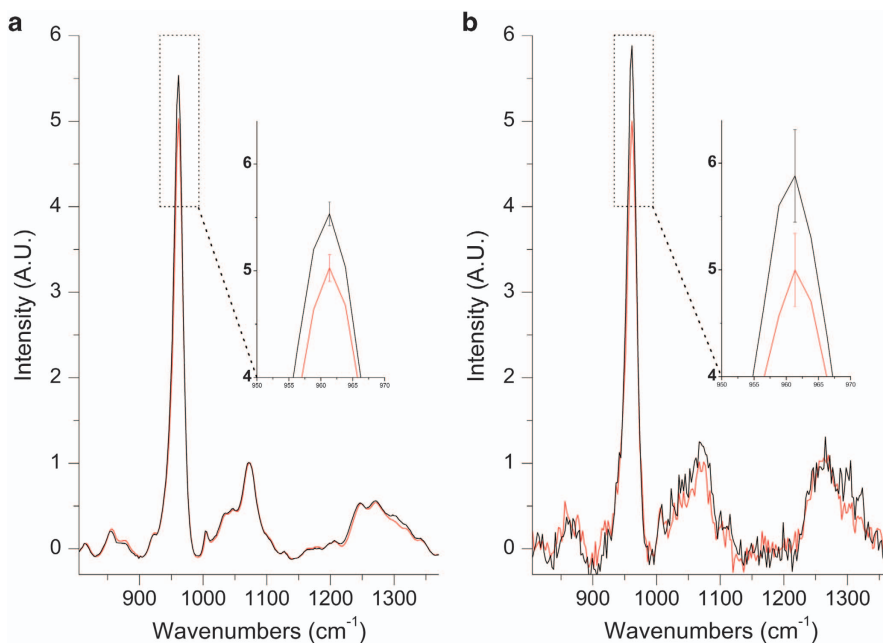


Figure 1 (a) A Raman spectrum of excised OI bone (black) and excised control bone (red). The inset shows mean \pm s.d. (b) A spatially offset Raman spectrum retrieved noninvasively through the skin of the same OI patient (black) and a spatially offset Raman spectrum retrieved noninvasively through the skin of an age-matched control (red). The inset shows mean \pm s.d.

examination). It is the first time a human bone disease has been detected with the technique *in vivo*.

Although OI is relatively rare, the demonstration that it can be detected with SORS in a clinical setting is nonetheless significant. The bone density of patients with some forms of OI can be normal, and, because X-ray-based diagnostic techniques cannot resolve the protein component of bone, these conditions can be very difficult to diagnose, especially in children. There have been cases where the authorities have mistakenly ascribed repeated bone fractures to child abuse, when the child in question was in fact an undiagnosed sufferer of mild OI.

The demonstration that SORS can detect a compositional abnormality noninvasively points the way to studies of bone diseases that have large societal impacts. For example, it is known from studies of excised specimens of bone that women with and without osteoporotic fracture have bone compositional differences that can be detected with Raman spectroscopy.¹² A technique that allowed the monitoring of these compositional differences *in vivo* could improve clinicians' ability to predict fractures and enable management strategies that would enhance subsequent quality of life.

Conflict of Interest

The authors declare no conflict of interest.

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