Raman Spectroscopic Measures of Bone Quality and Function

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Raman spectroscopy provides mineral and matrix composition information that are important inputs into bone quality (1). Raman spectroscopy can be performed on cells and fresh tissue as well as on fixed and embedded specimens and even on human subjects and live animals. Cell or tissue cultures can be followed over time.

With standard Raman microscopy instruments the spatial resolution is 0.5-1 micron. Low definition mapping and higher definition imaging are possible. In maps or images contrast is based on band intensities or on derived parameters such as band intensity ratios. With fiber optic probes non-invasive Raman spectra can be obtained on humans or animals at depths below the skin exceeding 1 cm, and in favorable cases 2 cm. Low definition two-and three-dimensional mapping has been demonstrated. However, validation of Raman markers of bone composition is just beginning (2), while validation is well-advanced in Fourier transform infrared (FTIR) spectroscopy. Additionally, the differing physics of Raman scattering and infrared absorption means that relative band intensities differ between the two methods of vibrational spectroscopy, especially for the mineral components.

Non-invasive spectroscopy and imaging are attractive for animal studies (3), because they allow measurements on the same animals over time. Our laboratory is currently investigating applications to autograft osseointegration and fracture healing in rodents. The same techniques can be used to monitor, for example, the effects of pharmaceuticals or diet on changes in composition. It is necessary to use custom-made fiber optic probes designed to account for the extensive turbidity of tissue. With such probes, low resolution mapping and even Raman tomography have been demonstrated (3). There have been brief preliminary reports of non-invasive bone Raman spectra from cadavers and even a live subject, but systematic studies have not yet appeared.

We have also shown that there are composition differences in the undamaged tissue from hip replacement subjects who suffered an osteoporotic fracture and tissue from the same site of controls (cadaveric) matched for gender, age and bone volume fraction who died from causes unrelated to osteoporosis or other bone disorders. As part of a larger study of several physical/chemical techniques, an ongoing study is testing the hypothesis that Raman spectra contain fracture risk as well as diagnostic information.

With polarized light Raman microspectroscopy can provide independent orientation and orientation distribution information on bone mineral and bone matrix orientation (5,6). Excised specimens are needed, but no special instrumentation is required. Such information supplements the composition information available from Raman spectroscopy and may be especially important for evaluation of biomechanical properties of bone. However, Raman spectroscopy probes the exposed surface of the tissue specimen only, while biomechanical properties are determined by bone architecture and composition at all scales and throughout the depth of the tissue.

References:

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