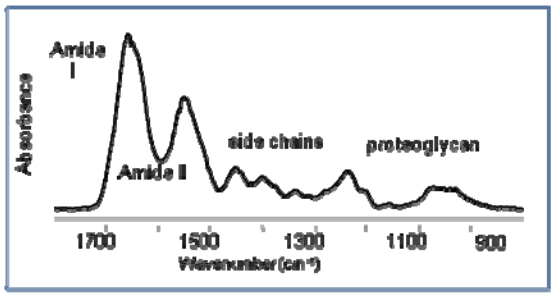
Optical Spectroscopy of Engineered Connective Tissues: Mapping Molecular Components

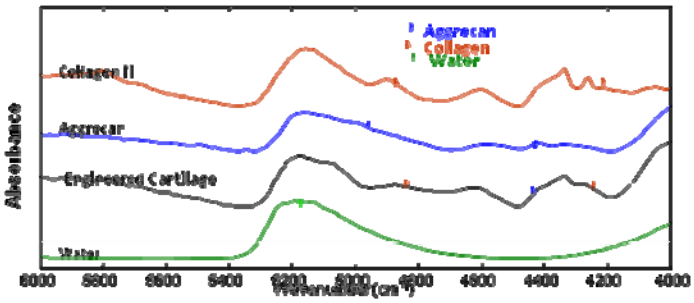
Nancy Pleshko, PhD, Exponent Inc., and Drexel University, Philadelphia, PA

Evaluation of the matrix composition of engineered constructs is a critical step towards optimization of tissue development. Magnetic resonance imaging (MRI), while offering non-invasive assessment of constructs, does not generally permit identification of molecular components of tissues. The use of optical spectroscopic methods as an adjunct to MRI offers the possibility of more specific mapping of molecular components in tissues, which could greatly facilitate the development of therapeutic interventions.

Extensive investigations over the past 10 years have established mid-infrared spectroscopy (mid-IR) as a viable approach for evaluation of musculoskeletal tissues, including bone and cartilage [1]. Mid-IR spectral analysis of bone, cartilage and skin enables identification of relative amounts of collagen, mineral, and proteoglycan, as well as assessment of molecular changes in protein integrity. In contrast to this established analytic method, the application of near infrared spectroscopy (NIRS) to connective tissue analysis is in the early development stage. NIRS uses shorter wavelength radiation than mid-IR and can therefore penetrate to a depth of millimeters-to-centimeters, as compared to 10's of microns. Therefore, non-invasive NIRS assessment of full-depth cartilage can be carried out under tissue engineering conditions as well as, and potentially, in the intact joint.



Mid-IR absorbances in cartilage arise from collagen (amide bands and side chains) and proteoglycan.

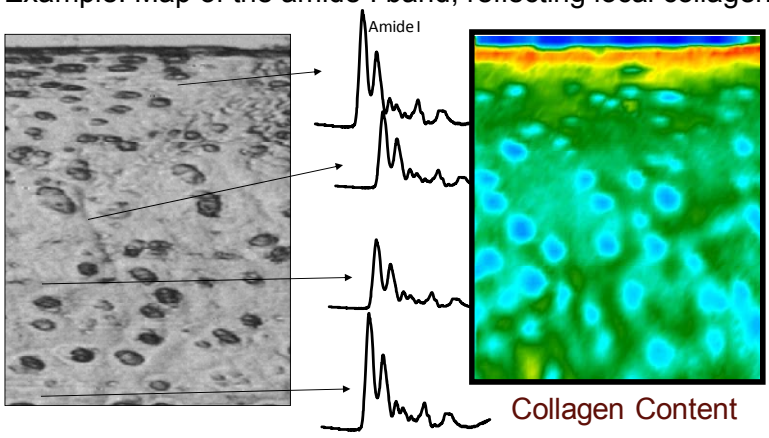


NIR absorbances in cartilage arise from collagen, proteoglycan, and water.

Spectroscopic Techniques for Assessment of Engineered Tissues:

1. Fourier transform infrared imaging spectroscopy, which we generally refer to as FT-IRIS, makes use of an array detector in conjunction with an optical microscope to produce a data set with an in-plane spatial resolution of 6.25 microns. A spectrum, reflecting local molecular characteristics, is assigned to each spatial location in the sample. Typically a thin (microns thick) tissue section is used, but thicker sections or intact tissues can be sampled as well. The data sets are used to create a spectroscopic image, which is a map indicating the strength of a particular absorbance band at each location within the sample.

Example: Map of the amide I band, reflecting local collagen content.



2. In Situ Mid-IR Spectroscopy: Evaluation of tissues in situ can be carried out using a mid-IR fiber optic probe [2,3], with degraded and normal cartilage being readily distinguished spectroscopically. These analyses are limited to evaluation of surface tissue properties due to the limited penetration depth of mid-IR radiation into tissue.

3. In Situ NIR Spectroscopy: The absorbance bands seen in the NIR region are overtone and combination bands of the mid-IR fundamental vibrations of C-H, C-O, O-H and N-H bonds [4], and are therefore appropriate for monitoring water, lipids, proteins and sugars. There are two primary limitations of NIR analyses. First, NIR overtone absorbances are at least 100-fold weaker than mid-IR fundamental vibrations, and second, NIR absorbances generally arise from combinations of molecular vibrations. Therefore, NIR data analyses generally require the use of multivariate data processing techniques. A further complexity is that photon scattering can have a substantial impact on the acquired spectra and must be accounted for.

Overview of Infrared Spectroscopic Techniques for In Situ Cartilage Analysis

	IR Imaging	Mid-IR Fiber Optic	NIR Fiber Optic
Sample Prep	Thin or thick sections; Intact tissues	None	None
Penetration Depths	1 - 10 microns	1 -10 microns	mm - cms
Spatial Resolution	6.25 microns	1 mm	1 mm
Data Output	Hyperspectral Image Data Set	Individual Spectrum	Individual Spectrum

References:

1. Boskey A, Pleshko Camacho N. Infrared imaging of native and tissue-engineered bone and cartilage. Biomaterials, 2007 May;28(15):2465-78.
2. Li G, Thomson M, DiCarlo E, Yang X, Nestor B, Bostrom MPG, Camacho NP . A chemometric analysis for evaluation of early-stage cartilage degradation by infrared fiber-optic probe spectroscopy. Appl.Spectrosc. 2005;59:1527-33.
3. Bi X, Yang X, Bostrom MPG, Bartusik D, Ramaswamy S, Fishbein KW, Spencer RG, Camacho N. Fourier transform infrared imaging and MR microscopy studies detect compositional and structural changes in cartilage in a rabbit model of osteoarthritis. Analytical and Bioanalytical Chemistry, 2007; 387(5):1601̃1612
4. Ciurczak EW. Near-Infrared Spectroscopy in Pharmaceutical Applications. In: Burns D.A., Ciurczak E.W, eds. Handbook of Near-Infrared Analysis. 2001:609.