Non-invasive Assessment of Engineered Cartilage Constructs Using Near Infrared Spectroscopy

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Introduction: A significant impediment to the success of tissue engineering strategies is the inability to fully assess the structure of developing tissue. Non-invasive assessment would permit appropriate ongoing intervention to modify the tissue towards desired structural and compositional endpoints. Extensive work over the past 10 years has established mid-infrared spectroscopy (mid-IR) as a viable approach for cartilage evaluation [1]. A limitation to this approach is that the penetration depth of mid-IR radiation into tissue is only ~10 microns, so that its use is restricted to surface evaluation. In contrast, near infrared spectroscopy (NIRS), which uses higher-frequency radiation than mid-IR, penetrates to a depth of millimeters-to-centimeters. It therefore holds the potential for non-invasive assessment of engineered cartilage constructs. Here we describe studies that demonstrate the potential of NIRS for non-invasive engineered tissue assessment.

Methods: Cell Culture: Articular chondrocytes from 2-4 week old bovine stifle joints were seeded into 0.27% collagen type I gel yielding a concentration of 2.5 x 106 cells/ml. The disc-shaped constructs were incubated in 6 well plates in serum-supplemented media at 37°C. Samples were grown over time, with and without mechanical input in the form of ultrasound, to create constructs with a range of material properties and matrix composition. Constructs conditioned with mechanical input were exposed to pulsed low intensity ultrasound (PLIUS) for either 20 mins or 40 mins, once per day, 5 days per week, at an intensity of 30mW/cm2 and a frequency of 1.5 MHz, with a pulse burst frequency of 1 kHz and burst duration of 200μs.

Infrared spectroscopy: A Nicolet Continuum FT-IR Microscope (Thermo Fisher Scientific Inc., Waltham, MA) was used to acquire NIR and mid-IR data. Full thickness cartilage constructs were dried at room temperature. IR data was acquired in the spectral region of 800-7000 cm\(^{-1}\) which spans both mid-IR and NIR frequencies. Proteoglycan (PG) sulfate absorbance was assessed using the 850 cm\(^{-1}\) spectral region [2], and structural water was assessed using the NIR peak at 5190 cm\(^{-1}\) [3].

Multivariate Analysis: The Unscrambler (CAMO Software, Oslo, Norway) was used for data processing using partial least squares (PLS) regression. For the current study, the NIR region was defined as 3800-5300 cm\(^{-1}\). A multiplicative scattering correction was applied to first derivatize NIR spectra, and the spectra were first regressed against number of weeks grown in culture. A full cross validation method was applied utilizing 69 samples from 4 different time points (1-4 weeks). The second set of models tested whether the NIR spectra could be used to estimate the proteoglycan content of the constructs using the mid-IR peak area of the 850 cm\(^{-1}\) PG band, and the frequency of 1.5 MHz, with a pulse burst frequency of 1 kHz and burst duration of 200μs.

Results: The NIR PLS1 model with 3 principal components was able to predict number of weeks in culture of the engineered cartilage samples with an estimated mean error of 0.38 weeks (R\(^2\)=0.89) as shown in Figure 2.

![Figure 2. Predicted # weeks vs. actual # weeks in culture](image)

A NIR PLS1 model with 4 principal components was used to predict the area of the 850 cm\(^{-1}\) PG band, R\(^2\) of 0.76, (Figure 3).

![Figure 3. Predicted PG peak area vs calculated PG peak area (850 cm\(^{-1}\))](image)

Finally, the area of the NIR water peak at 5190 cm\(^{-1}\) correlated with the 850 cm\(^{-1}\) PG peak (p<0.0001) (Figure 4).

![Figure 4. Area of the NIR water peak at 5190 cm\(^{-1}\) vs area of the PG peak at 850 cm\(^{-1}\).](image)

Discussion: These data demonstrate the potential for full-depth NIRS assessment of cartilaginous tissue in situ. Water and PG content can be elucidated from NIR spectra using both univariate (peak areas) and multivariate (PLS) methods. Further studies will address whether NIR spectra can also predict collagen content. In summary, these data are supportive of the further development of the NIR modality for non-invasive engineered tissue evaluations.

Acknowledgements: These studies were supported by NIH EB000744 and AR056145 (NP).