Near infrared for non-destructive testing of articular cartilage

Afara, I.,[1] Sahama, T.,[2] and Oloyede, A.[1]

[1]School of Engineering systems, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia.
[2]Computer Science Discipline, Faculty of Science and Technology, Queensland University of Technology, Brisbane, Australia.

Abstract. The concept of non-destructive testing (NDT) of materials and structures is of immense importance in engineering and medicine. Several NDT methods including electromagnetic (EM)-based e.g. X-ray and Infrared; ultrasound; and S-waves have been proposed for medical applications. This paper evaluates the viability of near infrared (NIR) spectroscopy, an EM method for rapid non-destructive evaluation of articular cartilage. Specifically, we tested the hypothesis that there is a correlation between the NIR spectrum and the physical and mechanical characteristics of articular cartilage such as thickness, stress and stiffness. Intact, visually normal cartilage-on-bone plugs from 2-3yr old bovine patellae were exposed to NIR light from a diffuse reflectance fibre-optic probe and tested mechanically to obtain their thickness, stress, and stiffness. Multivariate statistical analysis-based predictive models relating articular cartilage NIR spectra to these characterising parameters were developed. Our results show that there is a varying degree of correlation between the different parameters and the NIR spectra of the samples with R^2 varying between 65 and 93%. We therefore conclude that NIR can be used to determine, nondestructively, the physical and functional characteristics of articular cartilage.

Introduction

The mechanical properties of articular cartilage such as compressive stiffness have been used to assess its functional integrity in both normal and degraded conditions [1, 2]; and have been reported to successfully track changes in its structural integrity following controlled proteoglycan removal programs [3]. In addition, a number of researchers [4, 5] have suggested that an alteration of the mechanical properties in an arthritic joint may be notable before any gross morphological change is apparent. More recently, it has been noted that the mechanical stiffness of normal and degraded articular cartilage overlap to a significant degree [6]. In this paper we argue that despite this overlap, the mechanical parameter is still

useful if comparison is restricted to a normal and its equivalent degraded counterpart in an evaluation process.

The accuracy of these mechanical properties is significantly dependent on an accurate determination of the tissue's thickness. This relationship between cartilage thickness and stiffness was earlier noted by Hayes et al [7]. Given that cartilage thickness is site-dependent [8], this means that it is essential to determine tissue thickness at each test site when determining sample stiffness. Furthermore, while indentation test has been widely used for in vitro [9] and in vivo [10] assessment of the functional integrity of cartilage, an accurate and non-destructive method of measuring cartilage thickness in vivo remains challenging [9], thus necessitating this research.

To this end, we hypothesize that there is a substantive degree of statistical correlation between cartilage NIR absorbance spectrum, thickness and mechanical/functional characteristics. Near infrared spectroscopy is based on absorption of the NIR light resulting from chemical bond vibrations due to hydrogen and some light atoms. These bonds, C-H, N-H, O-H and S-H, are the predominant bond types in biological tissues like cartilage. In testing this hypothesis, we carried out experimental determination of cartilage thickness, stress-strain curves and associated parameters, and multivariate statistical (using partial least square regression - PLSR) analyses as described below.

Materials and Methods

Sample Preparation

Visually normal and intact bovine patellae, (N=15), harvested from prime oxen within 24 h of slaughter, were used in this study. The samples were wrapped in 0.15M saline soaked towels and stored at about -20^0C until required for testing. Prior to NIR spectroscopy, the intact patellae were thawed in 0.15M saline at room temperature for about 4 hours, then cartilage-on-bone blocks (n=173, lxbxh = 7x7x5mm) were extracted from the patellae. All tests were conducted with the specimens fully submerged or hydrated in 0.15M saline.

NIR Spectral Measurement

Diffuse reflectance near infrared spectroscopy was performed using a Bruker MPA FT-NIR (Fourier Transform NIR) spectrometer (Bruker Optics, Germany). The NIR light used spans the 800 - 2500 nm wavelength region; which is the near infrared region of the electromagnetic spectrum. The probe used consisted of 100 x Ø600µm fibres, with 50 transmitting and 50 receiving NIR reflected light. Using a holder that enabled x-y adjustment of the mounted sample, the probe was lowered to the specimen surface and firmly locked in position for each measurement (Fig 1a). After taking a reference spectrum, spectral data was obtained over the full

range of the NIR spectrum (Fig.1b), with each spectrum averaged over 64scans at 16 cm^{-1} resolution.

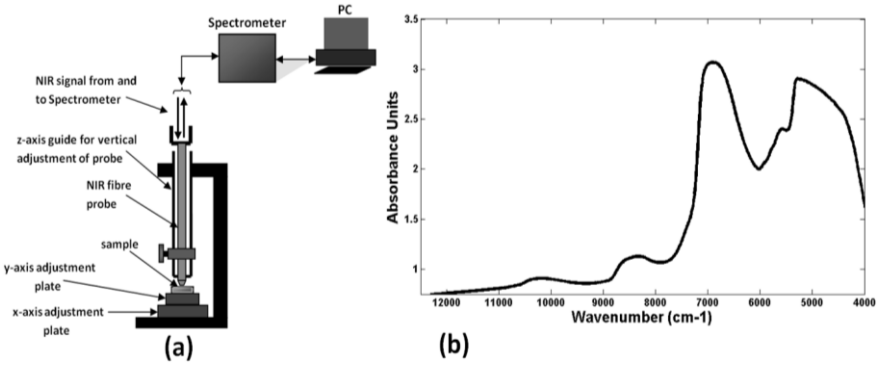

Figure 1.(a) Experimental setup (b) Typical NIR absorbance spectrum for normal intact cartilage

Mechanical Indentation Test

Each specimen, already set in dental acrylic cast, was placed in a holder and subjected to compressive loading on an Instron material testing machine (Instron, Norwood, USA) to 30% strain at a loading rate of 0.5mm/min. The load was applied via a 3mm plane-ended cylindrical indenter in the centre of the sample area. After indentation, the specimen was unloaded and allowed to recover for about 2hr in saline to ensure that full thickness has been regained before further tests were carried out. The stress–strain characteristic of the specimen was obtained from the load–displacement curve and its stiffness calculated as the slope of the tangent to the curve at nominated strains corresponding to zero, shoulder and asymptotic positions of the curve.

Thickness Measurement: Needle-probe Method

After load-displacement tests, the indenter was changed to a “needle-probe” one and the speed of indentation was set to 10 mm/min [11]. Cartilage thickness was measured by using the load cell to sense the instant when the needle touched the articular surface and when it contacts the calcified zone. The characteristic of the curve as the needle travels from the surface to the tidemark was used to determine sample thickness. Six measurements were taken at points lying within the regions under which the NIR spectrum and mechanical characteristics were obtained.

Results and analysis

The load–displacement curve of each sample was converted to its stress–strain characteristics using the sample thickness and indenter cross–sectional area. The stiffness at toe, shoulder and asymptote regions were calculated (Fig 2(a)).

The stress values of the shoulder (σ_s) and asymptote (σ_{as}) – stress at 30% strain were also obtained for correlation with the NIR spectrum. 97 samples of the total 173 samples were subjected to needle-probe thickness measurement. A more representative value of each sample thickness was evaluated as the average of six measurements at points lying within the regions under which the NIR spectrum and mechanical characteristics were obtained. The samples' thickness value ranged from *2.422 ± 0.033* to *1.337 ± 0.055* (mean ± SD).

Using the OPUS 6.5 software (Bruker Optics, Germany), calibration and validation was performed using partial least squares (PLS) regression on the spectra with scatter correction and spectral pretreatment. The scatter correction technique employed was multiplicative scatter correction (MSC), and first derivative pretreatment was used for spectral pretreatment. Leave-one-out (LOO) cross-validation method was used in the calibration process to determine the optimal number of PLS components and to estimate the performance of the developed calibration models.

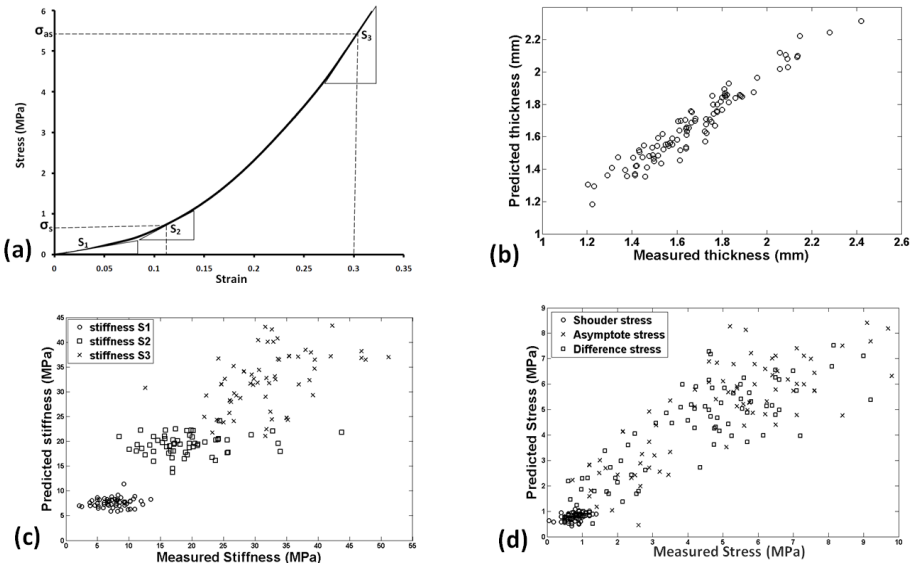

Figure 2. (a) Stress-Strain characteristic of articular cartilage showing points where the stiffness values were calculated. (b) NIR-predicted cartilage thickness versus measured thickness. (c) NIR-predicted cartilage stiffness versus measured stiffness. (d) NIR-predicted cartilage stress versus measured stress.

In cross-validation, calibration models are subsequently developed on parts of the data and iteratively tested (used for prediction) on other parts. Here, the model with the lowest number of components giving the highest R^2 (and possibly the lowest RMSECV) was selected. It should however be noted that the regions of the NIR spectrum that showed saturation due to O–H bond absorption of the NIR light were excluded from the analysis.

	Parameters	***Adjusted R^2 (%)***	***RMSECV***
Stiffness	*S1*	6.219	2.41
	S2	1.305	6.25
	S3	9.822	6.67
Thickness		93.02	0.0621
Stress	σ_s	19.39	0.219
	σ_{as}	65.46	1.33
	σ_{as} - σ_s	66.04	1.24

Table I. Physical and mechanical parameters of articular cartilage and their correlation with the NIR spectrum.

Discussion and Conclusion

Since articular cartilage is composed of constituents possessing functional groups such as C–H, O–H, N–H, and S–H which are selective absorbers of NIR radiation, an interaction between these constituents within the matrix structure and their resistance to compression may be reflected on the NIR spectrum of the tissue. Therefore, if there is any linear relationship between NIR spectrum and the structure of articular cartilage, calibration equations or models could be developed to predict structure– and function–based parameters of the tissue. Consequently, partial least squares (PLS) regression analysis results, shown in Fig. 2(b) – (d) and Table I, shows that the NIR spectrum relates strongly with the tissue thickness and, and almost unrelated to its stiffness.

Indicated by the significant scatter in the stiffness calibration plot shown in Fig. 2(c) and the considerably low correlation coefficient (Table I), the weak correlation between the stiffness parameters S1, S2, S3 and the NIR spectrum can be attributed to the significantly large overlap between the individual stiffness values of normal samples as observed by Brown et al [6]. Even though the current study did not consider degraded articular cartilage samples, our results confirm the hypothesis that stiffness parameters, used on their own, may not be reliable indicators of the tissue's functional viability. However, the asymptote stress (σ_{as}),

and the difference in stress values (σ_{as} - σ_s), Fig. 2(d), show better correlation with the NIR spectrum (see Table I), relative to the stiffness parameters. This resistance to compression may be considered as a more reliable parameter for assessing the tissue's functional viability.

The NIR–thickness calibration plot, in Fig. 2(b), shows a significantly high correlation (see Table I) between the NIR spectrum and the tissue thickness. While the NIR light pathlength through biological tissues has been shown to influence the resulting absorption spectra [12], the cartilage thickness can be likened to this NIR light pathlength through the tissue.

These results show that the NIR spectrum of cartilage can be used to estimate both the physical and functional characteristic of the tissue. In addition to being a non-destructive, real-time and faster alternative to conventional compression test, the flexibility of NIR makes it well suited for clinical application in the assessment of joint conditions. We therefore conclude that NIR, as a non-destructive method, can be used to assess the tissue condition with respect to its physical and functional characteristics.

References

[1] Harris, E.D.J., Parker, H.G., Radin, E.L., and Krane, S.M., (1972), *Arthritis and Rheumatism*, vol. 15, p. 497-503.
[2] Kempson, G.E., Spivey, C.J., Swanson, S.A.V., and Freeman, M.A.R., (1971), *Journal of Biomechanics*, vol. 4, p. 597-609.
[3] Lyyra, T., Arokoski, J.P.A., Oksala, N., Vihko, A., Hyttinen, M., Jurvelin, J.S., and Kiviranta, I., (1999), *Physics in Medicine and Biology*, vol. 44, p. 525-535.
[4] Appleyard, R.C., Ghosh, P., and Swain, M.V., (1999), *Osteoarthritis and Cartilage*, vol. 7, p. 281-294.
[5] Arokoski, J., Jurvelin, J., Kiviranta, I., Tammi, M., and Helminen, H.J., (1994), *International Journal of Sports Medicine*, vol. 15, p. 254-260.
[6] Brown, C.P., Crawford, R.W., and Oloyede, A., (2007), *Clinical Biomechanics*, vol. 22, p. 843-848.
[7] Hayes, W.C., Keer, L.M., Herrmann, G., and Mockros, L.F., (1972), *Journal of Biomechanics*, vol. 5, p. 541-551.
[8] Li, G., Park, S.E., DeFrate, L.E., Schutzer, M.E., Ji, L., Gill, T.J., and Rubash, H.E., (2005), *Clinical Biomechanics* vol. 20, p. 736-744.
[9] Suh, J.-K.F., Youn, I., and Fu, F.U., (2001), *Journal of Biomechanics*, vol. 34, p. 1347-1353.
[10] Lyyra, T., Jurvelin, J., Pitkanen, U., Vaatainen, U., and Kiviranta, I., (1995), *Medical Engineering and Physics*, vol. 17, p. 395-399.
[11] Jurvelin, J.S., Rasanen, T., Kolmonen, P., and Lyyra, T., (1995), *Journal of Biomechanics*, vol. 28, p. 231-235.
[12] Gussakovsky, E. and Kupriyanov, V., (2008), *Applied Spectroscopy*, vol. 62, no. 6, p. 671-676.