Measurements of ligament and cartilage optical properties at 351nm, 365nm and in the visible range [440-800nm].

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Abstract

To further evaluate the potential for intra-articular phototherapy, the optical properties of normal porcine ligament and cartilage were determined in vitro. The diffuse reflectance, Rd, was measured with an integrating sphere at 351, 365nm and in the range 440-800nm. The lateral spread of light introduced by a 400mm optical fiber was measured and analyzed to yield the optical penetration depth, δ. The two measurements, Rd and δ yielded the absorption coefficient μ_a [cm^{-1}], and the reduced scattering $\mu_s'=\mu_s(1-g)$ [cm^{-1}] at 351, 365nm and in the range 440-800nm.

keywords: optical properties, diffuse reflectance, in vitro, cartilage, ligamentf

1. Introduction

Rheumatoid arthritis is a common chronic inflammatory disorder involving the synovium (synovial membrane) of joints. It is characterized by the presence of three pathobiological phenomena: inflammation, abnormal cellular and humoral immune response and synovial hyperplasia. Over time, patients are disabled due to a progressive joint destruction. Current treatments focus either on systemic immune suppression due to the autoimmune nature of the disease, or on local surgical synovectomy which involves local ablation of the inflamed synovial tissue. However, because these available therapies are only partially successful, new strategies are being investigated.

Local therapy directed at the synovium offers the advantage of freedom of systemic side effects and the potential for synergy with systemic treatments, despite the limitation that multiple joints cannot be

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treated silmutaneously. A new local treatment of rheumatoid arthritis based on intra-articular PUVA phototherapy is being investigated, after preliminary studies by Laing et al. showed the feasibility of this technique.[1,2]

Due to the invasive nature of the procedure, a 3D Monte Carlo code is currently being developed to study the light transport processes involved and to simulate the treatment within the complex geometry of the joint: the goal is to determine how to best deliver a relatively uniform and sufficient dose of UVA light to the diseased synovial membrane, while sparing other joint's tissues such as cartilage.[3] Because the synovium is a very thin membrane, determining the optical properties of backing tissues, such as ligament, is also essential to simulate light transport phenomena in joints.

We use a simplified model of the joint walls by assuming that they are constituted of, either cartilage or synovium backed by ligament. There have been very few studies reported in the literature about those tissues optical properties and none in the UVA range. In this paper, we present the optical properties of cartilage and synovium backed by ligament at 351nm, 364nm and in the white light range [440nm-800nm].

2. Materials

Three samples of ligament and three samples of cartilage backed by bone were harvested from fresh pig shoulder and foot joints, and their optical properties were determined in vitro.

The total reflectance was measured with a 10.16cm inner diameter integrating sphere. Light sources were a UVA argon laser (351nm and 365nm), and a white light QTH 100W lamp (440-800nm).

The lateral spread of normally incident light introduced by a 400μm emitting optical fiber in contact with the sample was measured with an optical multichannel analyzer. The reflected light, R(r) was collected at increasing distances from the source with a second 400μm optical fiber in contact with the sample.

3. Methods

R(r) measurements were analyzed to yield the optical penetration depth, δ, for both white light and UVA light. The data were fit with the expression of the two-source model developed by Farrel et al[4]. The radial distribution of diffuse reflectance of normally incident light is based on two isotropic point sources; a positive source is located one mean free path below the tissue surface and one negative image source above the tissue surface. The positive source represents a single scatter source in the tissue, and the height in z of the image source above the tissue surface is given by:

$$z = 3D + 4AD \tag{1}$$

where 3D is the path length [5] defined as,

$$3D = \frac{1}{(0.35\mu_a + \mu_s')} \tag{2}$$

Boundary conditions are included in the term A:

$$A = \frac{(1 + r_d)}{(1 - r_d)} \tag{3}$$

where r_d is defined by the following empirical expression, [6]

$$r_d = -1.440n_{rel}^{-2} + 0.710n_{rel}^{-1} + 0.668 + 0.0636n_{rel} \quad (4)$$

and,

$$n_{rel} = n_{tissue}/n_{air}. \quad (5)$$

The radial distribution of diffuse reflectance of normally incident light is given by the expression:

$$R(r) = 3D\left(\mu_{eff} + \frac{1}{r_1}\right)\frac{\exp(-r_1\mu_{eff})}{r_1^2} + (3D + 4AD)\left(\mu_{eff} + \frac{1}{r_2}\right)\frac{\exp(-r_2\mu_{eff})}{r_2^2} \quad (6)$$

where r_1 and r_2 are the distances from the two sources to the light collection point. Note that an arbitrary scaling factor and offset were introduced to fit a relative reflectance profile in arbitrary units.

The diffuse reflectance, Rd, was calculated from the measured total reflectance assuming a tissue refractive index, n_{tissue}, of 1.37. For a semi-infinite tissue, the total diffuse reflectance Rd approximately behaves as:

$$R_d = \exp(-7.8\delta\mu_a) \quad (7a)$$

where δ is the 1/e optical penetration depth:

$$\delta = \sqrt{\frac{D}{\mu_a}} \quad (7b)$$

Np is defined as the ratio:

$$N_p = \frac{\mu_s'}{\mu_a} \quad (7c)$$

By combining equations (7a) and (7b), Np can be written as:

$$N_p = \frac{-7.8^2}{3(-\ln(R_d))^2} - 1 \quad (7d)$$

The two parameters, Np and δ yielded the absorption coefficient, μ_a [cm^{-1}], and reduced scattering coefficient, $\mu_s' = \mu_s(1\text{-}g)$ [cm^{-1}]:

$$\mu_s' = \frac{N_p}{\delta\sqrt{3(N_p + 1)}} \quad (8)$$

$$\mu_a = \frac{\mu_s'}{N_p} \quad (9)$$

4. Experimental results

UVA

Three readings of the total reflectance at 351nm and 365nm were obtained for each sample of ligament and cartilage. The average diffuse reflectances versus wavelengths are shown in Figure 1 for the two tissues.

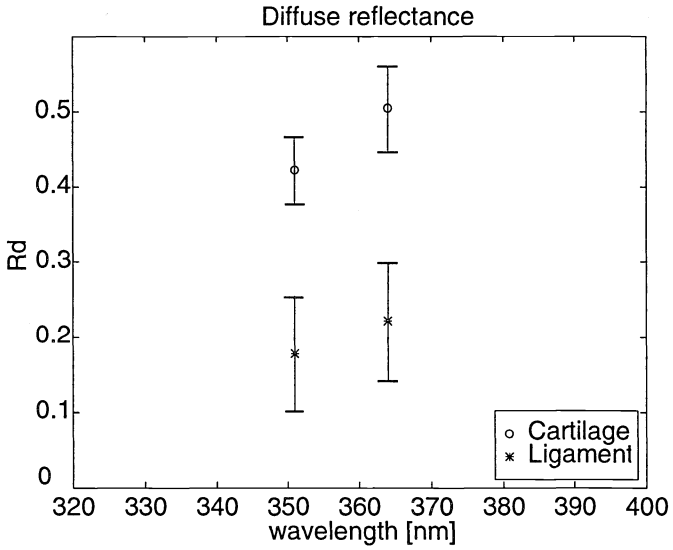

Fig. 1. Diffuse reflectance of cartilage (o) and ligament (*) at 351nm and 364nm.

Cartilage reflectances are about twice greater than that of ligament and although the errors for ligament reflectances are significant, the two can be easily differentiated. Reflectances measured at 365nm increase about 20 per cent compared to the ones at 351nm.

Diffuse reflectances were then combined with the R(r) data of each sample to obtain optical properties. Optical properties, mean and standard deviation, and the penetration depth δ, for ligament and cartilage, are summarized in Table 1.

Table 1: Summary of optical properties

Tissue	**Cartilage**		**Ligament**	
Wavelength	*351nm*	*364nm*	*351nm*	*364nm*
μa (cm^{-1})	3.6(0.73)	2.25(0.61)	5.7(1.7)	3.58(0.93)
$\mu s'$ (cm^{-1})	94(13)	88(13)	33(15)	29(14)
δ (cm)	0.03118	0.04084	0.04087	0.05548

Reduced scattering and absorption coefficients both decrease between 351nm and 365nm At both wavelengths, cartilage's scattering coefficients are greater than ligament's, while absorption coefficients are smaller. Cartilage and ligament penetration depths differ by a factor of 1.31 at 351nm and 1.36 at 365nm.

White light

Figure 2 shows the average diffuse reflectance spectra of ligament and cartilage in the range [440nm-800nm]. In addition, optical properties are presented in Figure 3.

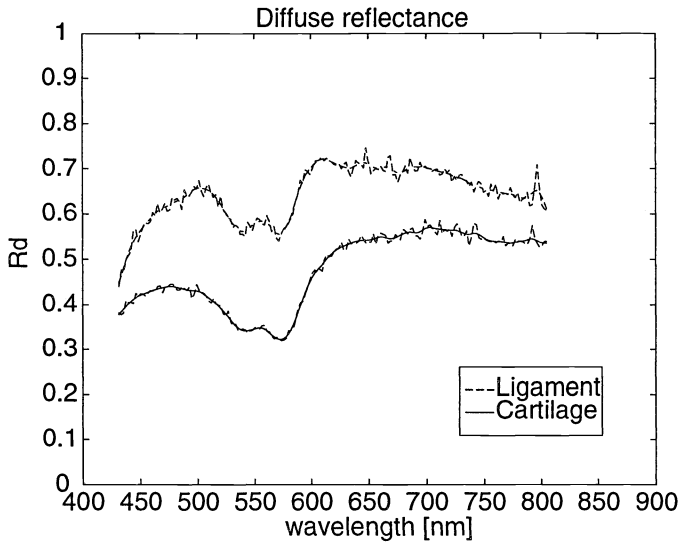

Fig. 2. Average diffuse reflectance of cartilage (solid line) and ligament (dashed line). Dotted lines show raw spectra. Spectra were smoothed with a cubic spline. The averages shown in this figure were obtained from the three samples spectra.

Average diffuse reflectance spectra show significant blood absorption in the range [440-630nm]. All spectra used to calculate the average also presented blood's characteristic absorption peaks. Cartilage reflectances are smaller than that of ligament.

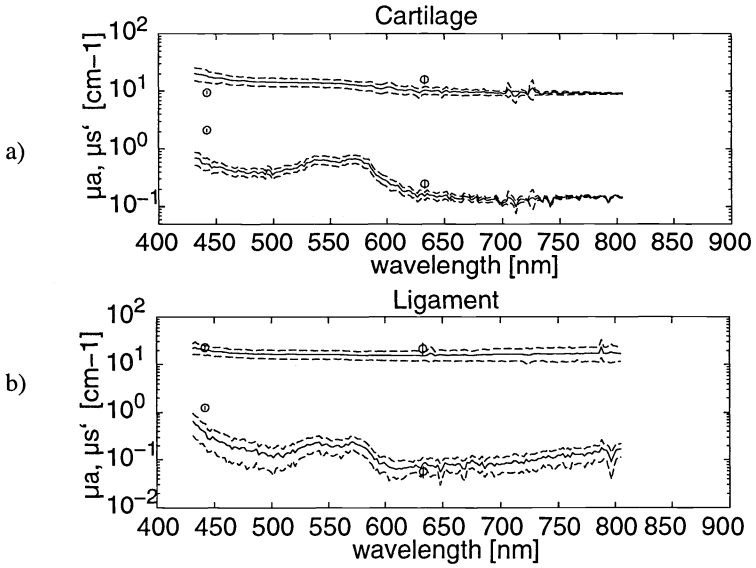

Fig. 3. Average optical properties of porcine cartilage (a) and ligament (b). In each plot, the upper curve represents the reduced scattering coefficient and the lower curve shows the absorption coefficient. Dotted lines represent the sample standard deviation. Rabbit cartilage and ligament optical properties (o) are plotted for comparison.

Cartilage scattering coefficient decreases with increasing wavelengths but the absorption coefficient slightly increases for wavelength greater than 725nm. Ligament's scattering coefficient decreased with wavelength for two out of the three samples. The absorption coefficient increases for wavelengths above 675nm. During previous experiments, we studied optical properties of cartilage and synovium backed by ligament harvested from rabbit knee joints, at 442nm and 633nm. Those results are presented next to the optical properties obtained during this study.

5. Discussion

The goal of the study was to determine the order of magnitude of cartilage and ligament optical properties at 351nm and 365nm as well as in the white light range.

In the near UV range, ligament diffuse reflectance readings presented significant variations increasing the error estimate of the ligament scattering coefficient at 351nm and 365nm. Cartilage diffuse reflectances, 0.42(0.04) at 351nm and 0.5(0.05) at 365 nm, and ligament reflectances, 0.18(0.07) at 351nm and 0.22(0.07) at 365 nm, are significant. But the geometry of the dilated joint cavity is complex, and we cannot count on achieving an "integrating sphere effect" during intra-articular PUVA treatment. Simulations will help define the required number of light source and their positions during treatment to achieve a relatively uniform dose at the synovium.

When comparing cartilage to ligament, almost half of the light escapes cartilage and its penetration depth, δ, is smaller by a factor of 1.31: UVA light will not travel as deeply in cartilage as it does in synovium backed by ligament. In addition, the concentration level of 8-MOP present in cartilage during PUVA treatment should be significantly smaller than that of synovium backed by ligament. Hyaline cartilage is an avascular tissue presenting a smooth surface of naked cartilage matrix. Embedded in the matrix, underneath the articular surface, chondrocytes get nutrients and oxygen, as well as 8-MOP, from the synovial fluid: the probability of 8-MOP diffusing to the chondrocytes is much lower than for a well perfused tissue. Therefore, PUVA treatment should have a limited impact on cartilage considering its optical properties and histology.

In the white light range, we observed a greater variation of ligament measurements than cartilage's. It may be due to the presence of small capillaries in the synovium lining the surface of ligament samples or to variations in synovium thickness.

The differences observed between cartilage optical properties of a rabbit patella at 442nm and 633nm and the results presented in this study are due to the very small size of the rabbit patella and to blood staining. Optical properties of synovium backed by ligament harvested in a rabbit knee joint at 442nm and 633nm match closely the optical properties presented in this paper, except at 442nm where the high absorption coefficient is probably due to blood absorption.

It is surprising that in the near UV range, cartilage scattering is higher than ligament and the absorption is smaller but the trend is reversed in the visible range. Ligament is composed of type I collagen fibers while hyaline cartilage consists in fine type II collagen fibrils distributed through an amorphous gel.[7] The smaller diameter of collagen fibrils in cartilage may be the reason of its increased short wavelength scattering compared to ligament. Further studies of the ligament and cartilage optical properties are needed in view of the results at 351nm, 365nm and in the with light range.

References

[1] Laing T. J., Ike R. W., Griffiths C. E., Richardson B. C., Grober J. S., Keroack B. J., Toth M. B., Railan D., Cooper K. D., "A pilot study of the effect of Oral 8-Methoxypsoralen and intraarticular Ultraviolet Light on rheumatoid synovitis," *J. Rheumatol.* **22**: 2126-31 (1995).

[2] Laing T. J., Richardson B. C., Toth M. B., Smith E. M., Marks R. M., "Ultraviolet light and 8-Methoxypsoralen inhibit expression of endothelial adhesion molecules," *J. rheumatol.* **22**: 2126-31(1995).
[3] Descalle M. A., Laing T. J., Martin W. R., "PUVA: a Monte Carlo code for intra-articular PUVA teatment of arthritis," *ANS Trans.* **75**: 141-142 (1996).
[4] Farrell T. J., Patterson M.S. Wilson B. C., "A diffusion theory model of spatially resolved, steady-state diffuse reflectance for the non-invasive determination of tissue optical properties in vivo," *Med. Phys.* **19**:879-888 (1992).
[5] Lin S. P., Wang L., Jacques S. L., Tittel F. K., "Measurement of tissue optical properties by the use of oblique incidence optical fiber reflectometry," *Appl. Opt.* **36**: 136-143 (1997).
[6] Groenhius R. A. J., Bosch J. J. T., Ferwerdo H. A., "Scattering and absorption of turbid materials determined from reflectance measurements, 1: theory," *Appl. Opt.* **22**: 2456-2462 (1983).
[7] Ham A. W., Cormack D. H., "Ham's histology," 9th ed, J. B. Lippincott Co., (1987).