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# Validation of Near Infrared Spectroscopy for Measurement of Water Content in Human Articular Cartilage Using Gelatin Model

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**Abstract**

This paper deals with the applicability of the near infrared (NIR) spectroscopy to quantitative measurement of the water content in human articular cartilage using gelatin model. We developed the device using NIR light at a wave length of 970nm, and investigated whether or not the device was useful for the measurement of the water content in articular cartilage. The gelatin was used as materials for the specimens with single, two- and three-layers. The absorbance defined as the intensity ratio between emitting and receiving NIR lights, the variation of receiving light intensity with the water content, and that with the distance between two probes of the light source and sensor were measured. The absorbance increased with increasing water content and with increasing probe distance ($R^2$=0.86~0.91). In the layered specimens, the light was supposed to pass through the surface layer and then reach the adjacent layer. These results showed that the device was useful for the measurement of the water content in the single and layered specimens. This study suggested that the device developed in this study have some possibilities of acquiring the quantitative data of water content at any point from the surface of articular cartilage.

**Key words**

Biomechanics, Articular Cartilage, Water Content, Near Infrared Spectroscopy(NIRS), Absorbance

## 1. Introduction

In human articular joints, the articulating bone ends of diarthrodial joints are covered by a thin (1~6 mm), dense, translucent, white connective tissue called hyaline articular cartilage. The primary roles of the articular cartilage are to provide joints with near frictionless function and to help absorbing mechanical shocks.

Usually, articular cartilage is assumed to be biphasic material consisting of incompressive two phases, the interstitial fluid matrix and the porous solid matrix [1, 2]. The normal articular cartilage consists of the collagen fiber content from 15 to 22% by wet weight, the proteoglycan (PG) content from 4 to 7% by wet weight, and the remaining 60 to 85% of water, inorganic salts, and small amounts of other matrix proteins, glycoproteins and lipids. The articular cartilage is devoid of blood vessels, lymphatic channels, and neurological innervations. Furthermore, the cellular density in the articular cartilage is less than 10%.

Collagen fibrils and PGs form the structural networks and support the internal mechanical stress when the loads applied to the articular cartilage.

Water, the most abundant component of articular cartilage, is most concentrated near the articular surface (~80%) and decreases near-linearly with increasing depth. The concentration of water in the depth zone is approximately 65% [3].

The articular cartilage has only a limited capacity for repair and regeneration, and if subjected to an abnormal range of stress in osteoarthritis (OA) can undergo total failure. In OA, degenerative changes of articular cartilage could lead to abnormal tissue swelling and functionally inferior biomechanical properties. OA may also arise secondarily from insult to the intrinsic molecular and microscopic structure of the collagen-PG matrix. Therefore, the water content in degenerated articular cartilage becomes higher than normal articular cartilage.

The conventional diagnostic methods of OA are X-ray imaging and the arthroscopic palpation of articular surface. They are dependent on experience and subjectivity of clinicians extremely, and are not designed to detect or classify the early signs of cartilage degeneration and may merely detect cartilage degeneration at advanced, irreversible phase [4]. So, it is very important to easily and quantitatively diagnose initial change of articular disease over time.

Using the feature that the near infrared (NIR) light is absorbed by the biological tissue, various diagnostic methods and devices have been developed recently [5-7]. On the other hand, the NIR spectroscopic meter has been used for nondestructive measurement of the water content in some kind of materials. So, in our previous study [8], the water content in articular cartilage was successfully and quantitatively measured using NIR spectroscopic meter at a known cartilage thickness. However, various problems such as shallow penetration depth of the NIR light, being not able to reproduce the inclination of water content in human cartilage and so on were caused.

Therefore, the experimental device using the NIR spectroscopy has been newly developed in the present study so as to solve these problems. The gelatin was used as an alternative material to articular cartilage, and the NIR absorbance was experimentally determined to discuss the usefulness of the device.

## 2. Materials and Methods

### 2.1 Materials

The gelatin was used to investigate the usefulness of the device developed in this study because the water content in gelatin was able to be easily controlled or changed, and this was suitable for the device to confirm its applicability to articular cartilage. The scattering coefficient of gelatin is different from that of articular cartilage. However, such difference in the scattering coefficient could have no significant effect on any conclusion of the present study. In the present study, we prepared two kinds of specimens made of gelatin. One was the single layered specimen and the other was the multi-layered specimen, namely specimens composed of two- and three-gelatin layers.

The water content of the single layered specimen was controlled to be 60, 65, 70, 75 and 80% (in mass%). The thickness of single layered specimen was chosen to be 20mm, according to previous study [9]. Five specimens were tested in each water content.

The water contents of the multi-layered specimens were chosen to be 60/50, 70/60 and 80/70% (surface/bottom) for two-layered specimens, and 30/70/60 and 30/80/70% (surface/middle/bottom) for three-layered specimens, respectively. The water content of 30% in the three-layered specimens corresponds to that in the surface skin of the leg. The thickness of each layer in the two-layered specimens was 10mm while that in the three-layered specimens was 7mm. Three specimens were tested in each set of water content for the two- and three-layered specimens, respectively.

### 2.2 Experimental device

In our previous studies [8] we found the several problems concerning the device for measuring the water content in articular cartilage. So, we decided to newly develop another type of the device based on NIR spectroscopy (Fig.1). The new device was able to adjust the distance between light-emitting and -receiving probes. Putting the light-receiving probe into one of the five holes, the probe distance can be easily changed between 8 and 32mm at every 8mm. An NIR LED (LED) (REVOX, 99970) with a wavelength of 970 nm was used as a light source since it is well known fact that the NIR light with a wavelength of 970nm is selectively absorbed by the hydroxyl group. A phototransistor (PT) (KODENSHI, ST-1MLBR2-C) was used to receive LED light which passed through the specimen, and connected with the personal computer (PC) (Lenovo, 9703-A15) through the terminal block (Interface, TNS-6812) and the AD board (Interface, PEX-361216). The emission intensity of LED was properly adjusted by a variable resistor. The electrical current flowed when PT received LED light, and its value was displayed on PC screen.

### 2.3 Measurement principle

The NIR light emitted from LED scatters in the specimens, and is detected by PT in terms of electrical current. The NIR light reaches deeper area with increasing probe distance. That is, the optical penetration depth to the specimens can be changed by changing the probe distance, $l$, between LED and PT (Fig.2). The NIR absorbance, $A$, can be determined by Lambert-Beer's law given as Eq. (1)[6]:

$$A = \ln\left(I_0 / I\right) \tag{1}$$

where $I_0$ is the intensity of emitted light from LED, $I$ the intensity of transmitted light through the specimen detected by PT. According to Eq. (1), the NIR absorbance is considered to be in proportion to the water content in the specimen. Both $I_0$ and $I$ are actually given as electrical current, and $I_0$ can be determined as the electrical current detected by PT when the reflector is placed at a downward position of 15mm away from the probe.

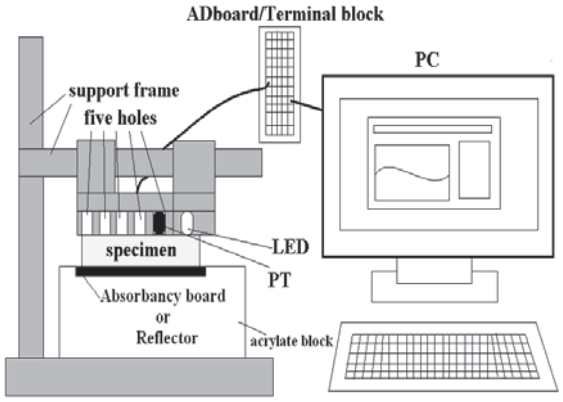


Fig.1 Schematic of experimental setup

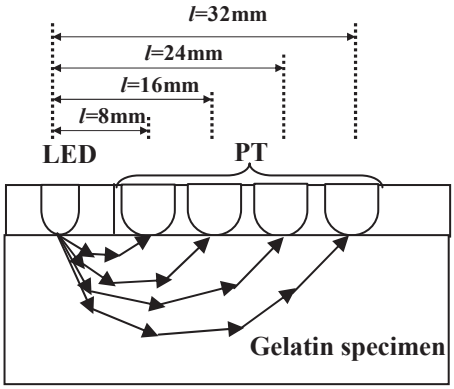


Fig.2 Schematic of change of NIR light paths in gelatin specimen with distance $l$ between LED and PT

### 2.4 Experimental method

LED light was irradiated to each specimen for about 30 seconds, and the NIR absorbance was calculated by using Eq. (1). The relation between the NIR absorbance and the water content was obtained, and then empirically formulated. To confirm the accuracy of this empirical formulation, the NIR absorbance was obtained from the specimen with an arbitrary water content. The calculated water content, $W_c$, from the formula was then compared with the measured water content, $W_m$.

Moreover, the NIR absorbance of the multi-layered specimens was measured, and compared with that of the single layered specimen.

## 3. Results

### 3.1 Single layered specimen

The results demonstrated that the intensity of transmitted light, *I*, exponentially decreased with increasing the measured water content, $W_m$, (Fig.3) and with increasing probe distance, *l*, (Fig.4). The absorbance, *A*, linearly increased with increasing the measured water content, $W_m$, ($R^2$=0.86~0.91) and with increasing probe distance, *l*, (Fig.5). From the result of Fig.5, we derived empirical formulas as a function of the absorbance, *A*, to predict the water content, $W_c$, in each probe distance (Table 1). The predictions of water content had good accordance with the measured water content ($R^2$=0.81) as shown in Fig.6.

### 3.2 Multi-layered specimen

At probe distance of 8mm, the two-layered specimens showed that the NIR absorbance, *A*, of the surface layer was almost same as that observed in the single layered specimens (Fig.7).

At probe distance of 8mm, the three-layered specimens showed that the NIR absorbance, *A*, of all three-layered specimens was larger than that of the single layered specimen with the water content, $W_m$, of 30% (Fig.8).

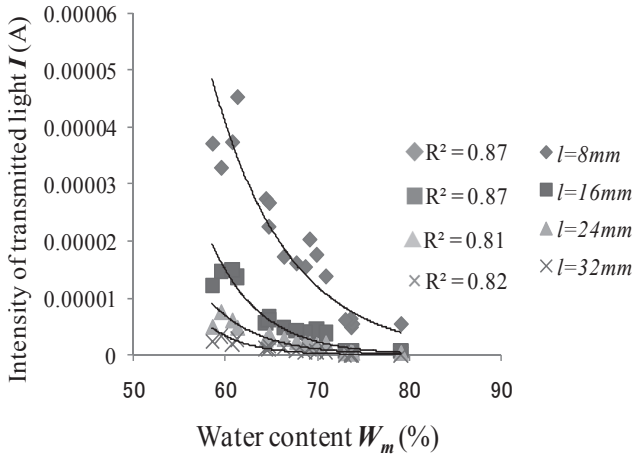


Fig.3 Relation between the measured water content $W_m$ and the intensity of transmitted light *I* for the single layered specimens with respect to the probe distance *l*. The intensity *I* is represented by electrical current

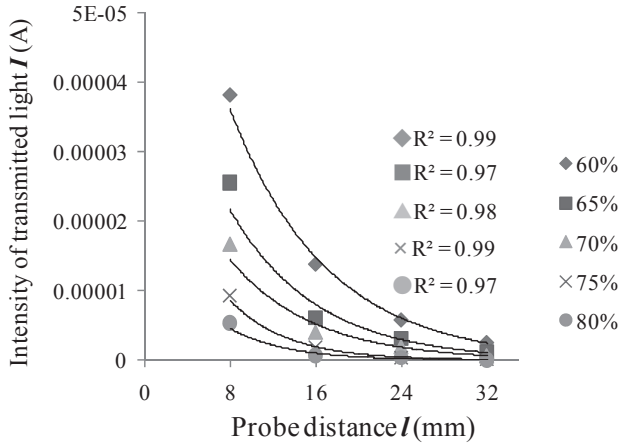


Fig.4 Relation between the probe distance *l* and the intensity of transmitted light *I* for the single layered specimens with respect to the measured water content $W_m$. The intensity *I* is represented by electrical current

Table 1 Formulas for the prediction of water content in the single layered specimens at each probe distance

| Probe distance *l* (mm) | Formulas for water content prediction $W_C$ (%) |
|---|---|
| 8 | $W_C = 8.78A + 42.58$ |
| 16 | $W_C = 6.14A + 45.02$ |
| 24 | $W_C = 6.11A + 43.38$ |
| 32 | $W_C = 5.00A + 45.79$ |

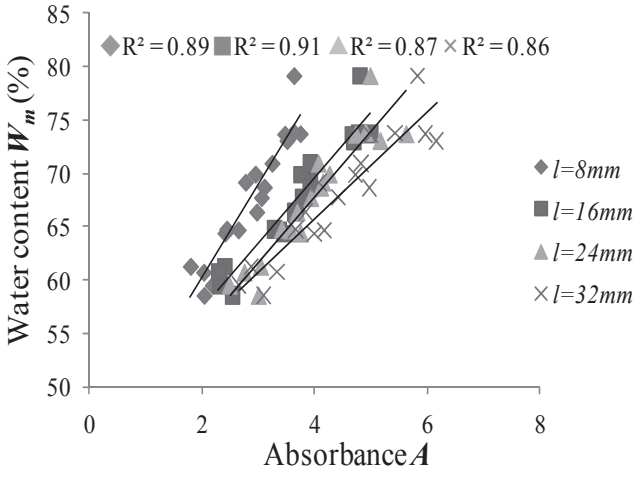


Fig.5 Relation between the NIR absorbance *A* and the measured water content $W_m$ for the single layered specimens with respect to the probe distance *l*

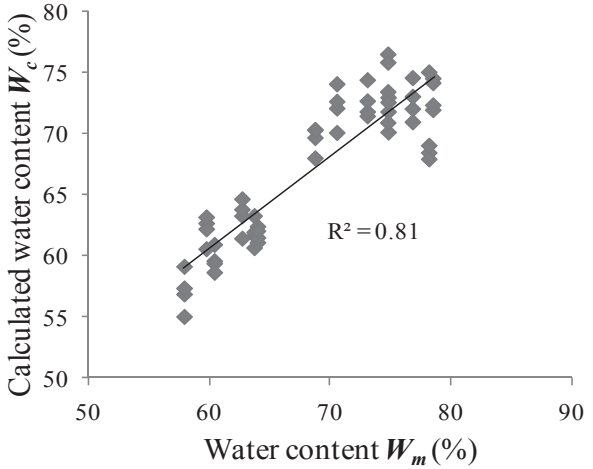


Fig.6 Relation between the measured water content $W_m$ and the calculated water content $W_C$ for the single layered specimens

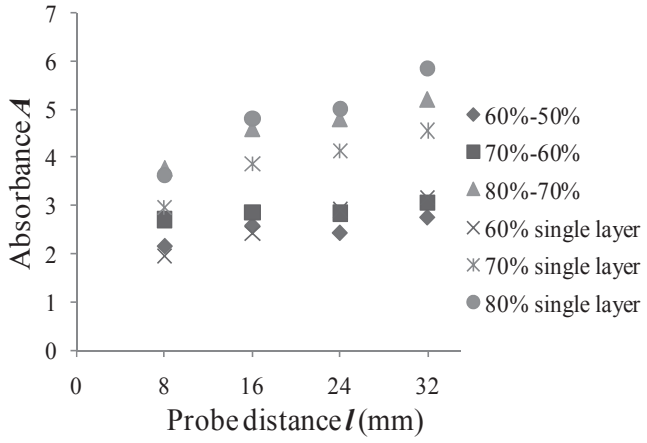


Fig.7 Relation between the probe distance *l* and the absorbance *A* for the two-layered specimens with respect to the measured water content. The results of the single layered specimens are also shown

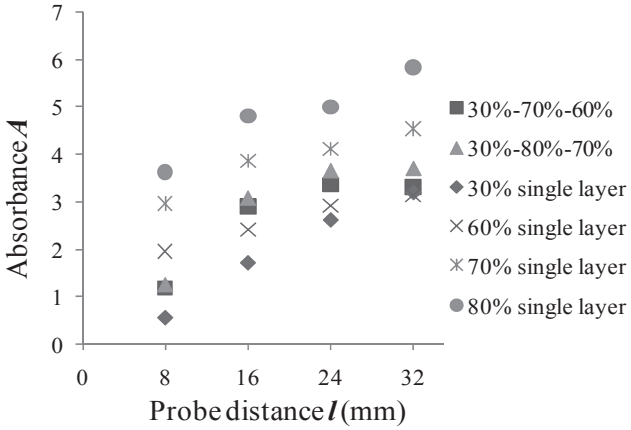


Fig.8 Relation between the probe distance *l* and the absorbance *A* for the three-layered specimens with respect to the measured water content. The results of the single layered specimens are also shown

## 4. Discussion

### 4.1 Single layered specimen

In the single layered specimen, the intensity of transmitted light exponentially decreased with increasing the water content. This suggested that the NIR light was absorbed by the water molecule. In addition, the intensity of transmitted light exponentially decreased with increasing the probe distance as well. This supposed that the light path became longer and the light reached deeper area as the probe distance was larger. The water content calculated from the NIR absorbance was highly reproducible, and, therefore, the device developed in this study was useful.

### 4.2 Two-layered specimen

The two-layered specimen showed that the NIR absorbance at probe distance of 8mm was almost same as observed in the single layered specimen. This result suggested that the NIR light at probe distance of 8mm passed only through the surface layer and never went down in deeper layer (Fig.9).

But, the absorbance of 60/50 and 70/60% gradually decreased between probe distance of 16mm and 24mm. And also, 70/60% specimen showed lower absorbance than 60% single layer specimen at probe distance of 32mm. The light reflection at boundary between surface and bottom layer appeared to enhance intensity of transmitted light.

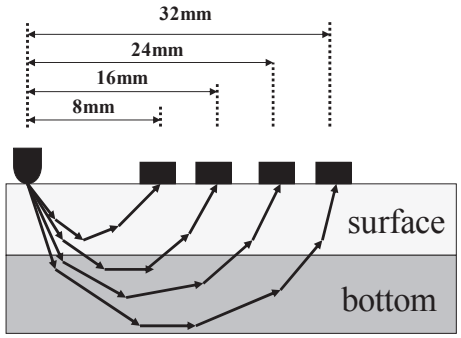


Fig.9 Expected optical path in the two-layered specimen

### 4.3 Three-layered specimen

The absorbance of the three-layered specimen was larger than that of single layered specimen with water content of 30% independently of the probe distance. The NIR light was supposed to pass through the surface layer and then reach the adjacent layer (Fig.10). In the layered specimens, the optical paths at the probe distances between 16 and 32mm were supposed to pass through both middle and bottom layers.

The absorbance of 30/70/60% gradually decreased between probe distance of 24mm and 32mm. The light reflection at boundary between surface, middle and bottom layer appeared to enhance intensity of transmitted light. Gelatin specimens without any boundary inside are considered to be required for further discussion.

The relation between the probe distance and the optical penetration depth has to be further discussed, and such relation could help us to determine the water content at arbitrary depths. Consequently, it can be said that the possibility of the quantitative measurement of the water content in articular cartilage was shown through the experiments of this study.

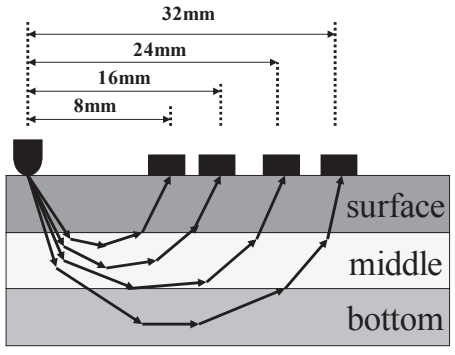


Fig.10 Expected optical path in three-layered specimen

## 5. Conclusion

The new device based on NIR spectroscopy has been developed for measuring the water content in gelatin specimens. The linear relation between the water content and the NIR absorbance at given probe distance was found and successfully formulated. The potentiality of the device as a quantitative and noninvasive diagnosis for measuring the water content in articular cartilage was consequently established through this formulation. Although further investigation concerning the miniaturization of the device as well as the relation between the probe distance and the penetration depth of NIR light should be required, clinical application of the device to degenerated human articular cartilage due to osteoarthritis is highly expected.

**Nomenclature**

$A$ NIR absorbance
$I_0$ Intensity of emitted NIR light, A
$I$ Intensity of transmitted NIR light, A
$l$ Distance between light-emitting and -receiving probes, m
$R$ Relative coefficient
$W_c$ Calculated water content, %
$W_m$ Measured water content, %

**References**

[1] Mow, V.C., Kuei, S.C., Lai, W.M. and Armstrong, C.G.: Biphasic Creep and Stress Relaxation of Articular Cartilage in Compression: Theory and Experiments, *J. Biomech. Eng.*, **102** (1980), 73-84.

[2] Mow, V.C. and Hung, C.T.: Biomechanics of Articular Cartilage, *Basic Biomechanics of the Musculoskeletal System*, Lippincott Williams & Wilkins, (1989), 31-58.

[3] Mow, V.C. and Lai, W.M.: Mechanics of Animal Joints, *Annual review of Fluid mechanics*, **11** (1979), 247-288.

[4] Outerbride, R.E.: The Etiology of Chondromalacia Patellae, *J. Bone and Joint Surgery*, **43**-B-4 (1961), 752-757.

[5] Eikje, N.S., Ozaki, Y., Aizawa, K. and Arase, S.: Fiber Optic Near-infrared Raman Spectroscopy for Clinical Noninvasive Determination of Water Content in Diseased Skin and Assessment of Cutaneous Edema, *J. Biomedical Optics*, **10**-1 (2005), 014013.

[6] Wilson, B.C. and Adam, G.: A Monte Carlo model for the Absorption and Flux Distribution of Light in Tissue, *Medical Physics*, **10**-6 (1983), 824-830.

[7] Arimoto, H., Egawa, M. and Yamada, Y.: Depth Profile of Diffuse Reflectance Near-infrared Spectroscopy for Measurement of Water Content in Skin, *Skin research technology,* **11** (2005), 27-35.

[8] Satou, S., Hamada, K., Tanabe, Y., Sakamoto, M., Kobayashi, K., Kikuchi, T. and Koga, Y.: Measurement of Water Content in Articular Cartilage Using Near Infrared Spectroscopy Meter. -Attenuation Characteristics of Near Infrared Ray- (in Japanese), *Japanese Society for Clinical Biomechanics* **29** (2008), 81-85.

[9] Durduran, T., Choe, R., Yu, G., Zhou, C., Tchou, JC., Czerniecki, BJ. and Yodh, AG.: Diffuse opticalmeasurement of blood flow in breast tumors, *Optics Letters*, **30**-21 (2005), 2915–7.