Spectrocolorimetric evaluation of human articular cartilage

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Summary

Objective: The aim of this study was to investigate whether human articular cartilage can be quantitatively evaluated using a spectrocolorimeter.

Materials and methods: Human articular cartilage specimens were analyzed using a spectrocolorimeter after macroscopic evaluation using the Outerbridge classification. The cartilage characteristics were examined, the L*, a*, b* colorimetric system, the spectral reflectance distribution and the yellow/red spectral reflectance percentage (Y/R SRP). Moreover, the results of the spectrocolorimetric evaluation were compared with the histological score described by Mankin et al.

Results: There were significant differences among the macroscopic four grades in the L*, a* and Y/R SRP values. The spectral reflectance distribution of grade 1 cartilage exhibited a gradual increase in the spectral reflectance ratio as the wavelength increased. The spectral reflectance curves of grades 2 to 4 cartilage had dips at a wavelength of around 580 nm. Across all the measured wavelengths, there were lower reflectance ratios with the progression of cartilage degeneration. Moreover, correlations were observed between the spectrocolorimetric values and Mankin score. A strong relationship existed between Mankin score and the Y/R SRP values.

Conclusions: The present study is the first to clearly demonstrate the relationship between spectrocolorimetric evaluation and the degeneration of human articular cartilage. The spectrocolorimeter may be a new quantitative evaluation tool for articular cartilage with clinical potential.

Key words: Osteoarthritis, Cartilage evaluation, Color, Spectrocolorimeter.

Materials and methods

PREPARATION OF CARTILAGE SPECIMENS

We examined 79 human articular cartilage specimens retrieved from patients who underwent total knee arthroplasty (TKA). From February 2007 to June 2008, 40 patients (8 males and 32 females; average age, 71 years; range, 51–83 years) were diagnosed with osteoarthritis and underwent TKA at the Department of Orthopedic Surgery, Nara Medical University. Pre- operatively, all the patients provided informed consent and agreed to have their excised articular cartilage evaluated using a spectrocolorimeter. During the TKA procedures, articular cartilage and bone were cut for insertion of the prostheses. The excised cartilage was washed with saline to remove blood and debris, and kept moist. Cartilage samples from the medial and lateral posterior condyles of the femur (2 cartilage specimens per patient) were used for this study. One sample was excluded from the series because it was destroyed by a bone saw while trimming the knee joint for TKA.

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MACROSCOPIC ANALYSIS

The 79 cartilage specimens were graded using a modification of the Outerbridge classification as follows: grade 1, softening and swelling of the cartilage; grade 2, fragmentation and fissuring in <50% of the measurement area of the spectrocolorimeter; grade 3, fragmentation and fissuring in >50% of the measurement area of the spectrocolorimeter; grade 4, erosion of cartilage down to the bone. The center (4 mm in diameter) of each articular surface of excised posterior condyle was identified as the measurement area (Fig. 1). Each of the specimens was evaluated separately and independently by three trained orthopedic surgeons. Two observers independently graded the specimens. If their results were the same, the grade was adopted. If their results differed, the third observer decided the grade of the specimen.

SPECTROCOLORIMETRIC MEASUREMENTS

Details of the spectrocolorimetric examination have been described elsewhere6. Briefly, a commercial spectrocolorimeter (X-Rite SP64; X-Rite K.K., Tokyo, Japan) was used for cartilage color assessment. The measurement conditions were as follows: reference illumination, D 65 (standard daylight); geometry, d/8; incident light, diffuse; observation angle, 10°; specification methods of color, L*, a*, b* colorimetric system and spectral reflectance distribution of the object’s color. With regard to the L*, a*, b* colorimetric system, the L* (luminance) value measures brightness ranging from black (0) to white (100), while the a* value expresses the color spectrum from green (–) to red (+) and the b* value expresses the color spectrum from blue (–) to yellow (+)7,8. Regarding the other index of cartilage color evaluation, the spectral reflectance distribution was automatically calculated at 10-nm wavelength intervals from 400 to 700 nm. The X-Rite SP64 had a measurement area of 4 mm in diameter. To calibrate the instrument, a standard white plate and black trap were used. The X-Rite SP64 was positioned with minimal pressure perpendicular to the cartilage surface of excised posterior condyle was identified as the measurement area and stained with hematoxylin and eosin and safranin-O-fast green. Sections were scored by an orthopedic surgeon under blinded conditions according to the histological grading scale composed four categories described by Mankin et al. and were assigned a score ranging from 0 to 14 points. A high total score represented severe cartilage degeneration.

HISTOLOGICAL EVALUATION AND SCORING

After the spectrocolorimetric evaluation, the cartilage samples were fixed in 10% formalin, decalciﬁed in ethylenediaminetetraacetic acid (EDTA), and embedded in parafﬁn. The section were prepared from center of the measurement area and stained with hematoxylin and eosin and safranin-O-fast green. Sections were scored by an orthopedic surgeon under blinded conditions according to the histological grading scale composed four categories described by Mankin et al. and were assigned a score ranging from 0 to 14 points. A high total score represented severe cartilage degeneration.

STATISTICAL ANALYSIS

All data in this study are reported as means ± standard deviations. For multiple comparisons, the groups were analyzed using the non-parametric Kruskal–Wallis test. When significant variance was demonstrated, differences between individual groups were determined using the Mann–Whitney U-test with the Bonferroni correction9. The relationship between spectrocolorimetric data and the histological data were analyzed using the non-parametric Spearman’s rank-order correlation method. Statistics used to compare the spectrocolorimetric data by macroscopic grade and to analyze the relationship between spectrocolorimetric data and histological data were used on referring to book and previous studies6,10. Statistical analyses were performed using Excel Statistical Program File Ystat 2008 (developed by Yamazaki S, Igakutosyo syuppan Co., Ltd., Tokyo, Japan). In all analyses, the significance level was set at P < 0.05.

Results

On the basis of the Outerbridge classification, eight samples were grade 1, 28 were grade 2, 24 were grade 3 and 19 were grade 4. The samples were divided into four groups (grades 1 to 4) and the L*, a*, b* values [Fig. 2(A–C)], spectral reflectance distributions (Fig. 3) and Y/R SRP values [Fig. 2(D)] for each group were calculated.

Fig. 1. Human cartilage samples excised from the posterior condyle of the femur. The cartilage samples were graded macroscopically using the Outerbridge classification as grade 1 (A), grade 2 (B), grade 3 (C), grade 4 (D). Open circles (4 mm in diameter) indicate the measurement area of macroscopic, spectrocolorimetric and histological assessment.
The $L^*$ values were $74.6 \pm 4.4$ for grade 1, $72.9 \pm 3.6$ for grade 2, $68.6 \pm 4.0$ for grade 3 and $61.6 \pm 1.7$ for grade 4 [Fig. 2(A)]. There were significant differences among the four groups ($P < 0.01$, Kruskal–Wallis test). However, by post hoc multiple comparison tests, no significant difference was detected between grades 1 and 2 ($P = 0.68$). The $a^*$ values were $1.7 \pm 0.9$ for grade 1, $2.9 \pm 1.7$ for grade 2, $4.4 \pm 2.2$ for grade 3 and $9.2 \pm 2.1$ for grade 4 [Fig. 2(B)]. There were significant differences among the four groups ($P < 0.01$, Kruskal–Wallis test). However, by post hoc multiple comparison tests, no significant difference was detected between grades 1 and 2 ($P = 0.47$). The $b^*$ values were $21.5 \pm 3.0$ for grade 1, $21.7 \pm 4.5$ for grade 2, $18.4 \pm 4.6$ for grade 3 and $19.5 \pm 3.2$ for grade 4 [Fig. 2(C)]. There were significant differences among the four groups ($P = 0.047$, Kruskal–Wallis test), but a significant difference was only found between grades 2 and 3 ($P = 0.048$) by post hoc multiple comparison tests.

Typical examples of the spectral reflectance curves for grades 1, 2, 3 and 4 are shown in Fig. 3. For grade 1, there was a gradual increase in the spectral reflectance ratio as the wavelength increased. For grades 2 to 4, the spectral reflectance curves had dips at a wavelength of around 580 nm. Across all the measurement wavelengths, there were lower reflectance ratios with the progression of cartilage degeneration.

The $Y/R$ SRP values were $88.7 \pm 4.8$ for grade 1, $82.4 \pm 5.7$ for grade 2, $72.8 \pm 7.3$ for grade 3 and $56.5 \pm 5.2$ for grade 4 [Fig. 2(D)]. There were significant differences among the four groups ($P < 0.01$, Kruskal–Wallis test). Moreover, there was also a significant difference between grades 1 and 2 by post hoc multiple comparison tests ($P = 0.012$).

The Mankin scores were $2.5 \pm 2.2$ for grade 1, $6.0 \pm 2.4$ for grade 2, $9.6 \pm 1.9$ for grade 3, $11.5 \pm 1.9$ for grade 4.

Relationships between spectrocolorimetric data and the histological data are shown in Fig. 4. There was a correlation between the Mankin score and the $L^*$ value ($rs = 0.76$), the $a^*$ value ($rs = 0.79$) and the $Y/R$ SRP values ($rs = 0.86$) from spectrocolorimetric examination. A strong negative relationship existed between the Mankin scores and the $Y/R$ SRP values.
In the present study, a spectrocolorimeter was found to be effective for judging cartilage degeneration using retrieved human cartilage specimens as a minimally invasive method. The main findings of the study were that cartilage degeneration can be detected by reductions in the $L^*$ and $Y/R$ SRP values and increases in the $a^*$ values. Moreover, cartilage degeneration exhibited a decrease in the spectral reflectance ratio at a wavelength of around 580 nm. These results are useful for classifying degenerated cartilage grade in a numerical manner.

Our study attempted to develop constructs for human cartilage assessment based on the $L^*$, $a^*$, $b^*$ colorimetric system and the spectral reflectance distribution. In the present study, significant decreases in the $L^*$ values and significant increases in the $a^*$ values were found with the progression of cartilage degeneration. The $L^*$ values express the luminance of the cartilage, while the $a^*$ values express the red/green of the cartilage color. Therefore, the reductions in the $L^*$ values imply that the cartilage surface loses its glossiness due to cartilage degeneration. Regarding the $a^*$ values, the observations differed between human and rabbit cartilages. In animal studies, translucent intact cartilage is affected by the blood color of the subchondral bone, owing to the thinness of the cartilage\(^5\). In human cartilage, the influence of the subchondral bone is small. Therefore, human cartilage exhibited higher $a^*$ values, according to the progression of cartilage loss.

There was a characteristic change of the spectral reflectance curve at a wavelength of around 580 nm. In other words, there was a decrease in the spectral reflectance ratio at a wavelength of around 580 nm with the progression of cartilage degeneration. We used this change to determine the cartilage lesion grades. We defined the decrease in the reflectance ratio at around 580 nm as $Y/R$ SRP. In our study, significant differences in the $Y/R$ SRP values were found among the four grades. Therefore, the $Y/R$ SRP values can provide diagnostically important information about human cartilage degeneration.

With regard to the relationships between spectrocolorimetric data and the histological data, the results show similar with that on the basis of Outerbridge classification. Namely, the $L^*$ and $Y/R$ SRP values decreased and the $a^*$ values increased with the progression of histological cartilage degeneration. Moreover, a strong negative relationship existed between the Mankin score and the $Y/R$ SRP values. Hence, $Y/R$ SRP values can be used to predict the histological change of articular cartilage.

In conclusion, this study reports the first data regarding the relationship between spectrocolorimetric evaluation and the degeneration of human articular cartilage. Analysis of the many factors that affect cartilage color is a very complex subject that requires much further investigation. Nevertheless, the $Y/R$ SRP values can be expected to become one of the quantitative indexes for human articular cartilage. Further studies on the construct validity of the $L^*$, $a^*$, $b^*$ colorimetric system and the spectral reflectance distribution for human articular cartilage are recommended.

**Conflict of interest**

The authors have no conflict of interest.

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