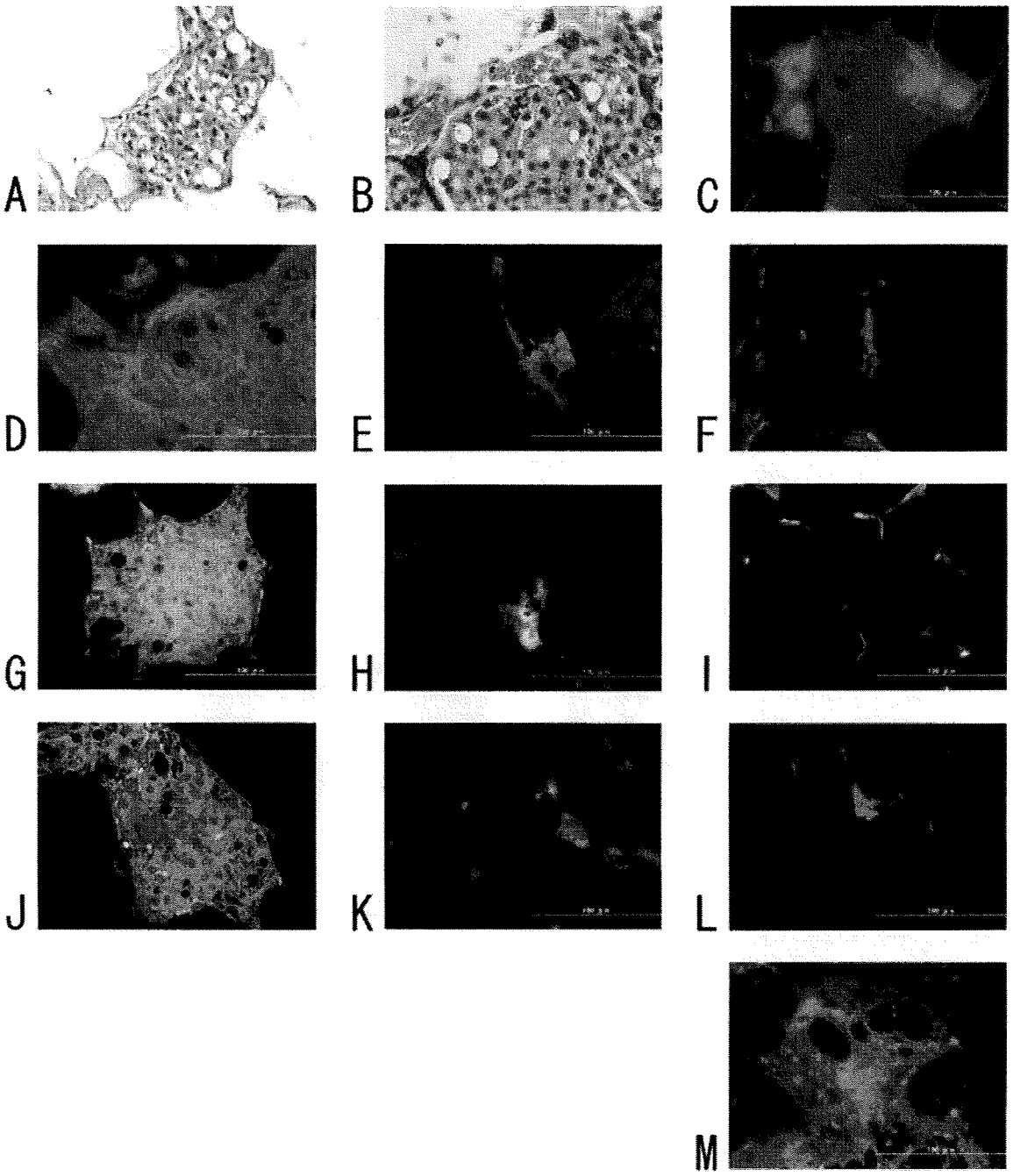

Fig. 4. Histological analysis of NPs at the transplant site of recipient rats. HE (A), safranin-O (B) and keratan sulphate IHC (C) staining indicate the presence of transplanted NPs at day 5. Safranin-O stains the proteoglycan of NPs (Red), and keratan sulphate is specific extracellular matrix of NPs (Green). Keratan sulphate was stained even 26 days after transplantation in the recipient of NOD mouse (M).

CD3 (D~F), CD68 (G~I) and CD161 (J~L) (Red) are immunohistochemically double-stained with keratin sulphate (Green). D, G, and J are the results at day 5; E, H, and K at day 10; F, I, and L at day 40. Keratan sulphate decreased dependent on time, NK cells and macrophages decreased simultaneously.

To summarize, the results of the *in vitro* CFI and cytotoxicity assays revealed the presence of a spleen cell population that had cytotoxic effects on autologous NP cells. This cytotoxic spleen cell population is composed of sub-populations of NK cells and macrophages. Furthermore, as a negative control of cytotoxicity assay, we used fibroblast-like cells of *annulus fibrosus* origin. The result was that the fibroblast-like cells was tolerant to isogeneous spleen cells, however *nucleus pulposus* cells was sensitive. This result raised our test hypothesis that NP is sensitive to specific immune cells.

***In vivo* study**

BLI study. We performed a BLI study to investigate immunological responses to transplanted NP tissues *in vivo*. The BLI evaluation showed a significantly higher survival rate for transplanted NP cells in the Lew-NOD group compared to that in the Lew-Lew group totally ($p = 0.036$), at day 7 (0.35 ± 0.18 *vs.* 0.11 ± 0.05; $p = 0.042$) and at day 21 (0.16 ± 0.08 *vs.* 0.03 ± 0.02; $p = 0.037$) (Fig. 3A). After 90 days, up to 13% of the transplanted NP cells had survived in the Lew-NOD group (Fig.3B). NP cells transplanted into Lewis rats (Lew-Lew) did not survive past 21 days, when luminescence at the NP cell transplant site had decreased to near background levels (Fig.3C).

IHC staining. Because our results showed that transplanted NP cell survival was reduced in association with an immunological reaction, we used immunological staining to identify which types of immune cells had infiltrated. Transplanted NP tissues in rats at day 5 existed mainly in the loose subcutaneous fat tissue as an agglomeration of cells with a bubble-like extracellular matrix (Fig. 4A). Safranin-O staining showed the presence of red-stained proteoglycans (Fig. 4B), and fluorescent green-stained keratan sulphate, both of which are constituents of the extracellular matrix of the NP (Fig. 4C). We observed transplanted NP tissues at day 5 (Fig. 4D, G, J), however, the amount of transplanted NP tissue was markedly decreased at day 10 in the Lewis rats, while NP tissue was obviously present in NOD mice recipients even at day 26 (Fig. 4M). From the IHC evaluation of immune cells in Lewis rats, no CD3 positive T cells attraction were observed subcutaneously from day 5 to day 40 (Fig. 4D, E, F). Attraction of NK cells and macrophages was observed at days 5 and 10 around the outgrown NP tissues (Fig. 4G, H, J, K); however, neither NP tissues nor immune cells were observed at day 40 (Fig. 4I, L). The numbers of NK cells and macrophages in microscope fields that included agglomerated NP cell clusters decreased with time (Table 2).

Discussion

The precise mechanism of immunological involvement in the pathology of disc herniation has not been defined. We performed two immunological assays, the CFI and the cytotoxicity assay, using co-cultured NP and immune cells. We also developed an *in vivo* subcutaneous transplantation model and measured the survival rate of transplanted NP cells using the BLI method. IHC at the transplant site of the recipient rats was used to identify the immune cells.

The suppression of NP cell colony formation was observed to be dependent on the effector:target (E:T) cell ratio. We found that non-adherent (CFU-NA) colonies were more strongly suppressed by immune cells than adherent (CFU-A) colonies. Because NP cells are known to be heterogeneous (Chelberg *et al.*, 1995), this difference in colony formation may reflect the different epitopes recognized by immune cells, or possible differences in the immune privilege function, like the presence or absence of Fas ligand. Based on these possibilities, about 20% of the NP cell population were alive even at E:T cell ratio of 100:1, which appears to differ immunologically from other NP cells. Of particular interest was the assessment of direct

Table 2 The number of immune cells in a microscope field (x40)

	Day 5	Day 10	Day 40
T cell	None	None	None
NK cell	2-5	1	None
Macrophage	4-7	1-3	None

This table indicates the number of representative agglomerated NP cell clusters in the tissue specimen of transplanted site in Lewis rats. NK and macrophage cells were observed in the transplanted site in the early phase, whereas T cells were not observed. In day 40, neither NP tissues nor immune cells were observed.

cytotoxic function of the immunological cell types. The results of the cytotoxicity assays of isolated T, NK, and macrophage cells demonstrated that only the NK and macrophage cells had cytotoxic activity on NP cells. The target molecules and their location on the NP cells remain undefined; further biological and immunological studies are necessary.

The results of the BLI study showed differences in the survival rate of NP cells in the transplanted NP tissues between the Lewis rat and NOD mouse recipients. NP cells are known to undergo apoptosis (Park *et al.*, 2001), and intervertebral disc cells are thought to be able to behave as competent phagocytes (Jones *et al.*, 2008). However, these results do not explain the different survival rates of NP cells in the current study. Because the survival rate of NP cells was higher in immunodeficient mice than in Lewis rats, immunological functions are implicated. NOD/shi-scid mice lack mature lymphocytes, and have macrophage dysfunction, a reduced level of NK cell activity and absence of circulating immune components compared to wild-type mice. These factors may account for the difference in NP cell survival rate between NOD mice and Lewis rats in our study.

We also detected the infiltration of specific immune cells into the NP transplant sites; these results definitively demonstrate the immunological activity of these cell types against NP tissues. Macrophages and NK cells, but not T cells, were detected, although the presence of T and B cells in isolated human herniated discs and in experimental porcine models has been previously reported (Geiss *et al.*, 2007). Our results suggest an early immunological response after normal NP tissues were exposed to the immune system. Thus, macrophages and NK cells were observed on days 5 and 10 when residual NP was present, but not on day 40 when the transplanted NP had disappeared. This finding supports the presence of an immunological response to transplanted NP tissues.

In our IHC study, CD68 positive cells did not resemble the appearance of resident chondrocytes. Although Jones *et al.* (2008) suggested that CD68 positive cells were transformed resident intervertebral disc cells, based on their morphology, the results of our study show that these macrophages are not transformed resident cells but rather are infiltrating cells.

Autologous tissues are generally not recognized as foreign by the immune system. The NP is an immune-privileged tissue isolated from the immune system (Hiyama *et al.*, 2008) and it, like similarly isolated tissues, including the eye and testis, can produce inflammatory autoimmune responses (Wildner and Diedrichs-Möhring, 2004; Schuppe and Meinhardt, 2005). Another possible trigger for autoimmunity is innate immunity, which is induced by chemical factors without specific antigen-antibody responses, leading to rapid immune responses to pathological microbe antigens. Because the NP cell produces chemical factors and the carbohydrate structure of the extracellular matrix produced by NP cells may mimic that of pathological microbe antigens, the NP may trigger an innate immunity response (Bárdos *et al.*, 2005).

In the *in vivo* transplantation model, we utilized a xenogeneic model because the mouse is too small to obtain enough donor NP cells. The use of NOD mice as recipients is well established for evaluating the effects of immunodeficiency. In addition, the xenogeneic transplantation model is commonly used for immunological evaluation (Yoshino *et al.*, 2000).

In conclusion, even non-degenerated NP cells elicit an immune response, and macrophages and NK cells in particular are shown to have an early immunological function when NP cells are exposed to the immune system. While these results may not be directly applicable to the human, this study provides important information for understanding the pathophysiological mechanism of disc herniation.

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Development of the hyperspectral cellular imaging system to apply to regenerative medicine

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ABSTRACT

Regenerative medicine by the transplantation of differentiated cells or tissue stem cells has been clinically performed, particularly in the form of cell sheets. To ensure the safety and effectiveness of cell therapy, the efficient selection of desired cells with high quality is a critical issue, which requires the development of a new evaluation method to discriminate cells non-invasively with high throughput. There were many ways to characterize cells and their components, among which the optical spectral analysis has a powerful potential for this purpose. We developed a cellular hyperspectral imaging system, which captured both spatial and spectral information in a single pixel. Hyperspectral data are composed of continual spectral bands, whereas multispectral data are usually composed of about 5 to 10 discrete bands of large bandwidths. The hyperspectral imaging system which we developed was set up by a commonly-used inverted light microscope for cell culture experiments, and the time-lapse imaging system with automatic focus correction. Spectral line imaging device with EMCCD was employed for spectral imaging. The system finally enabled to acquire 5 dimensional (x, y, z, time, wavelength) data sets and cell-by-cell evaluation. In this study, we optimized the protocol for the creation of cellular spectral database under biological understanding. We enabled to confirm spectrum of autofluorescence of collagen, absorption of specific molecules in the cultural sample and increase of scattering signal due to cell components although detail spectral analyses have not been performed.

Keywords: hyperspectral imaging, cellular function, intrinsic optical spectrum, regenerative medicine, cell sheet,

1. INTRODUCTION

Regenerative medicine is now developed to a practical level. The transplantation of differentiated cells or tissue stem cells has been clinically performed, particularly in the form of cell sheets and also engineered tissue. To ensure the safety and effectiveness of cell therapy and sheet transplantation, the efficient selection of desired cells with high quality is a critical issue, which requires the development of a new evaluation method to discriminate cells non-invasively. The required evaluation method should enable to discriminate cells in culture condition of monolayer or cell-sheet. There were many ways to characterize cells and their components, among which the intrinsic optical spectral analysis has a powerful potential for this purpose [1].

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There are several possible situations to discriminate cells using intrinsic optical spectra [2]. Cell cycle is one of key factors for the cell characterization. Different Cell phase, such as cell in S-phase and in M-phase has completely different cellular character. Chromatin is contributing factor in genomic change and cell cycle modulation. The microscopic observation of chromatin would be easily accepted as highly scattering substance in the nuclear. The scattering parameter of chromatin would be extracted depending on the cell cycle. Collagen, as major contents of extracellular matrix, is autofluorescence substance. The formation of pericellular matrix would be monitored, which directly related cell-sheets' function. Mitochondria would denote energy status of cell and relating NADH complex, which is well-known optical absorber. Therefore, quantitative optical parameters are potentially very helpful in determining the cellular character.

Our ultimate goal is to develop a total cellular imaging system demonstrating useful intracellular and/or extracellular spectrum including proper analyses, which enables to discriminate desired cells for cell safety and efficacy for application to regenerative medicine. That is, the intrinsic optical spectrum, which has a relation to cellular biological understanding, could indicate whether the cell can be practically applied to regenerative medicine.

2. BUILD UP HYPERSPECTRAL CELLULAR IMAGING SYSTEM

2.1 Hyperspectral Imaging

Hyperspectral imaging devices are common in remote sensing reconnaissance. It captures both spatial and spectral information in a single pixel. Hyperspectral imaging collects the same picture at different wavelength to generate a "datacube" that can reveal objects. Another advantage of this imaging form is that different element leaves unique spectral signatures behind in various bands of the spectrum. Using these specific signatures, it is possible to identify the materials that make up an observed object.

We developed microscopic hyperspectral imaging system in order to discriminate cells and demonstrated the optimal protocol to obtain the spectrum of arbitrary intracellular and/or extracellular region. The system was equipped with time-lapse microscopy and spectroscopic imaging apparatus including an EMCCD detector described details below (2.2 – 2.4). The protocol for spectral acquisition was determined for synchronizing the hyperspectral image with general microscopic image.

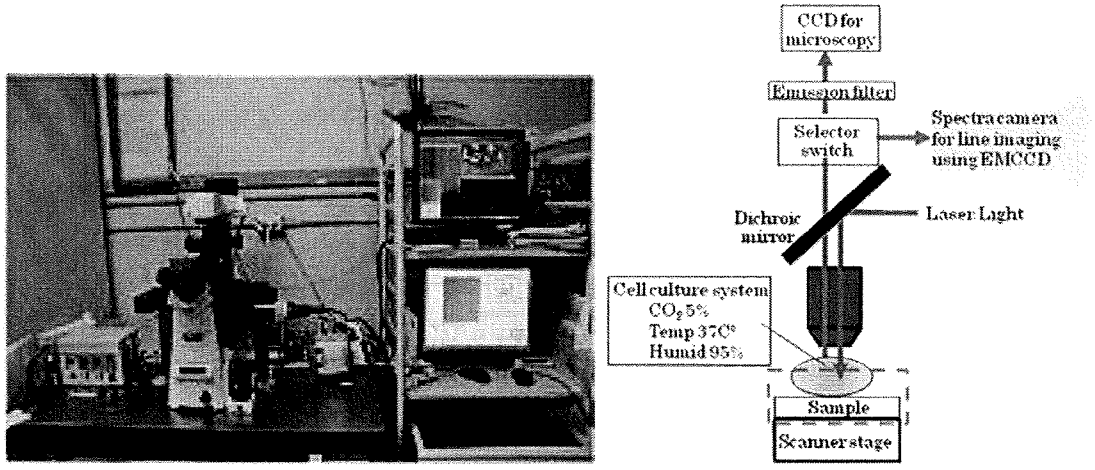

Fig. 1 Apparatus for the hyperspectral cellular imaging system (The system configuration in the photograph (left) refers to the drawing (right))

2.2 Components - 1. Time-lapse microscopy

Integrated flexible microscope system (Nikon, ECLIPSE Ti) which allowed high-speed screening image capture was employed. The microscope system has faster operation speed which could reduce overall light exposure. It was equipped with time-lapse imaging with automatic focus correction system and CO_2 incubator cell culture system. The time-lapse image enabled that the same object (e.g., a cell) is photographed at regular time intervals over several hours. The time-lapse serial data allow us to observe the morphological change in the process of differentiation and/or lineage conversion such as iPSC emergence which could be definitively confirmed by its shape [3].

2.3 Components - 2. Imaging spectrograph

ImSpector V8 (Specim) was employed as the imaging spectrograph in our system. It has visible range (380-800 nm) with 8 nm spectral resolution. It works as a spectral line imaging device which produces full contiguous spectral information in each line pixel with high spectral resolution to provide a push-broom hyperspectral operation.

2.4 Components - 3. EMCCD and Software

Andor's iXon^{EM}+DU-897 back illuminated EMCCD (Electron Multiplying Charge Coupled Device) was employed. Its active pixels were 512 x 512 and frame rate is 35 fps. The 'on-chip' amplification process of the EMCCD was realized without sacrificing the photon collection capability of the sensor, with back-illuminated sensors offering up to 95% Quantum Efficiency (QE). Thus, the system was equipped with a suitable sensor. Original software for the hyperspectral operation was developed linked to general microscopic image. The line scanned hyperspectral image were confirmed as identical system to the general microscopic image precisely.

Table 1 Feature of the developed system

Wavelength range	380~780nm
Spectroscopic tool	Spectral line imaging
Spectral resolution	8nm
Image resolution	X axis : 30μm/object lens magnification
	Y axis : microscopic view field/512 pixels
Imaging rate	35 frame/sec maximum
Noise reduction	Dark-current and thermal noise correction
Image processing Image display	-Color image display from spectrum to RGB conversion
	-Spectral display at each pixel
	-Image display at specific wavelength
	-Calculation function of color difference

3. PROTOCOL FOR DETERMININATION OF INDIVIDUAL SPECTRUM OF ARBITRARY INTRACELLULAR AND/OR EXTRACELLULAR REGIONS

The cell-related known spectra to date might not be proven because the spectra were obtained under undefined biological aspect. The spectra of cell components (nuclear chromatin, subcellular organelle and extracellular matrix) for regenerative medicine should be determined under biological realistic condition. Consequently, in order to reach our ultimate goal, spectral database should be created on biological understanding at the first step in order to ensure cell safety and effectiveness using the intrinsic spectrum of arbitrary intracellular and/or extracellular region. That is, proper protocol for the spectral acquisition should be designed. In this study, light source has not been optimized yet.

PROTOCOL

I. Baseline acquisition of the hyperspectral image for setting exposure time and gain after dark-current and thermal noise correction.
*Sample: culture dish filled with culture medium as background
II. General microscopic observation.
*Phase contrast image or differential interference contrast image
III. Determination of arbitrary intracellular and/or extracellular regions for spectral acquisition.
IV. Hyperspectral image acquisition under the set exposure time and gain.
V. Marked (stained) microscopic observation to visualize distribution the specific region (nuclear, subcellular organelle and extracellular matrix)
*Example: Hoechst stain (nuclear), Cell mask stain (whole cell), PKH stain (cell membrane)
VI. Determination of the particular spectrum for creation of database
VII. Spectral database creation under biological understanding condition

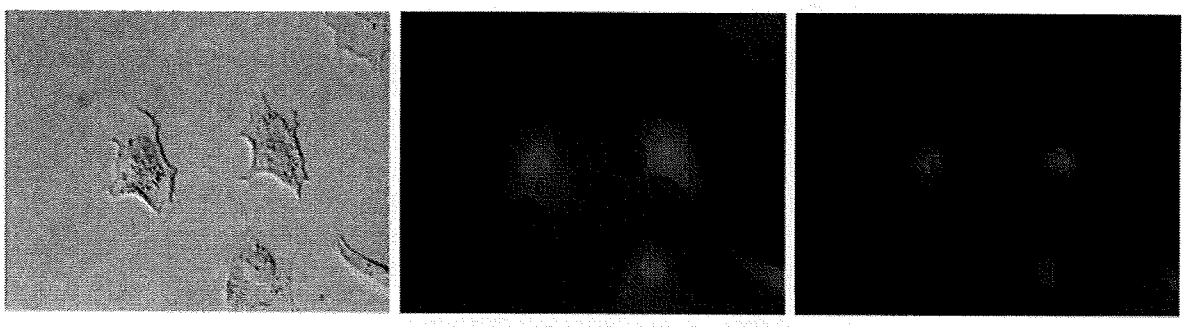

Fig.2 Primary cartilage cells obtained from a knee joint of Japanese White rabbit cultured for monolayer in an incubator with air containing 5% CO_2 at 37°C. A: differential interference contrast image B: image stained with Cell mask, C: image stained with Hoechst

4. CONCLUSION

We have successfully developed the hyperspectral imaging system for acquiring spectrum of cells itself used in regenerative medicine. The system finally allowed to acquire 5 dimensional (x, y, z, time, wavelength) data sets. We enabled to confirm spectrum of autofluorescence of collagen, absorption of specific molecules in the cultural medium and increase of scattering signal due to cell components (data not shown) although detail analyses have not been performed.

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新しい医療技術

軟骨再生医療に有効な光技術

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要旨：再生医療で重要な役割を果たす周辺工学技術の一つに，細胞・組織の機能を評価する計測技術が挙げられる。われわれは，生体に安全なレーザー光を用いて簡便に力学特性や細胞外マトリックスの性状を評価できる計測技術を開発している。組織工学的手法を用いて作製した軟骨を対象にした実験結果を基に軟骨再生医療の評価法として開発技術の有効性を検証した。

再生医療は失われた臓器・組織・器官の再生や機能の回復を目的とした医療で，先端医療として着目されており，典型的な融合境界領域として医学・生物学・工学の連携によって実現可能となる医療である。

再生医療で重要な役割を果たしている周辺工学技術に，計測・イメージング技術が挙げられる。再生した細胞・組織の形態と機能の評価は，再生組織の一部あるいはすべてをサンプルとして提供しないでできる非侵襲的方法で行う必要がある。同時に，評価すべきパラメーターとして再生医療の正当性や有効性を正確に判断するための指標を的確に選択する必要がある。的確な指標でないと，組織工学的手法を用いて組織を構築する過程における移植の至適なタイミングが決められない。

日常生活動作（ADL）を下げ，生活の質（QOL）の低下も招く変形性関節症をはじめとする運動器疾患の新しい治療として，軟骨再生医療が着目されている。軟骨の再生医療は1990年代前半から海外で実施・報告され，米国をはじめとする諸外国では既に2万例に近い手術症例の蓄積がある。しかし，その対象疾患は小さな軟骨の外傷性病変であり，再生医療が真に必要とされる変形性関節症の治療は未だ実施されていない。

関節軟骨の修復・再生に最適な組織工学的軟骨の開発が最重要課題であることは明らかであるが，同時に軟骨再生医療にとって重要な評価パラメーターを適切に評価することも軟骨再生医療実現のための重要課題である。軟骨再生医療では組織工学的手法で細胞外マトリックスの成熟を促し，力学機能を回復し再建させる。すなわち，重要な評価パラメーターの1つは力学特性を表すパラメーターとなる。また，関節軟骨の再生医療では，本来の硝子軟骨組織に再生されるのではなく，線維軟骨組織として再生されてしまうことがしばしば問題となっている。

こうした問題を未然に防止するためのモニタリ

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Optical technology in regenerative medicine of articular cartilage

Key words：Photoacoustic, Autofluorescence, Articular cartilage

ングとして，軟骨組織を構成するコラーゲンの自家蛍光の測定は，軟骨組織の生化学的組成に関する成熟度の指標となる可能性がある。軟骨再生医療の評価には形態情報も重要であるが，力学特性やコラーゲン組成に関する情報の方が，よりニーズを満たしたパラメーターとなる。

光/レーザー光技術は医療現場に優位に持ち込め，広く有効に活用できる。これは次のような特長を持つことによる。

- 経ファイバー的・経内視鏡的に生体へアプローチが可能
- 傷害性のない光・レーザー光を選択することで，非侵襲的な診断が可能
- 高い空間分解能，および，高い時間分解能の微細治療・診断が可能

光/レーザー光を用いると形態情報だけでなく，物理学的・生化学的な情報の取得も可能で，選択的かつ特異的な作用を可能にする。このような特長は軟骨再生医療の評価にも有用である。アメリカなどで研究開発が進んでいるMRIを用いた軟骨の評価方法や，日本やヨーロッパで臨床応用が開始されている超音波に比べて，レーザー光を用いる利点は，上記に挙げた点のほかに，一度に複数の情報が得られることと，MRIやX線などの画像診断装置と比べると非常にコンパクトで，さらに，簡便に取り扱うことができ，かつ即時に診断結果が出ることである。もちろん，放射線被曝，造影剤の使用や磁場の影響などのような侵襲性はない。

われわれは，パルスレーザー光照射により局所で発生した応力波が，組織内を伝播する過程で組織固有の力学特性により減衰する現象に着目し，光音響法で力学特性を計測できる基本原理を提案した。応力波の減衰時間（τ）が組織の粘性（η）と弾性（G）の比（η/G）に相当するという原理である。われわれは原理実証実験として，力学特性を変化させた生体ファントムを測定対象に，光音響法を用いて計測された粘弾性パラメーターである減衰時間と既存の侵襲的粘弾性分析装置から得られる物質固有の粘弾性特性値（tanδ）に相関があることを示し，提案した方法で力学特性が測定できることを実証した[1)2)]。さらに，細胞外マトリックスの主要成分の一つであるコラーゲンが自家蛍光物質であること，自家蛍光は生体内に内在する物質から発生する蛍光であるため生体内の性状を反映することから，自家蛍光による組織性状評価法について提案・検討した[3)4)]。軟骨組織には構成成分・機能が異なる硝子軟骨，線維軟骨，弾性軟骨があり，硝子軟骨の細胞外マトリックスはII型コラーゲンとプロテオグリカンが主要な成分で，線維軟骨の場合はI型コラーゲン優位の細胞外マトリックス，弾性軟骨の場合はコラーゲンとともにエラスチンを多く含んでいる。われわれは，光音響法で用いるレーザー光を自家蛍光の励起光とし，時間分解自家蛍光スペクトル測定により得られる波長と時間を関数とするパラメーターが，コラーゲンの分子種で異なることを自家蛍光特性実験で検証した。これを細胞外マトリックスの性状を評価する方法とし，光音響法と組み合わせた評価法を開発している。提案しているシステムを図1に示す[5)]。

まず，本開発システムの安全性を確認するために，細胞増殖活性試験においてレーザー光照射による軟骨への影響を確認した。細胞増殖活性試験には，Cell Counting Kit-8を使用するWST-8アッセイを用いた。培養した軟骨細胞にレーザー光を照射して，増殖活性試験試薬を滴下した後，37℃，5% CO_2気相の条件のインキュベータで培養し，上清の吸光度より細胞の増殖活性を算出した。結果を図2に示す[6)]。現在の測定条件としている50倍のショット数の場合でも，使用している装置の最大出力のエネルギーの場合でも，ネガティブコントロールとしたレーザー光を当てない細胞群と増殖活性に有意差はなかった。また，ポジティブコントロールとしたアルコール滴下群とは異なる増殖活性を有意差を持って示した。これにより，現在設定しているレーザー光の条件では細胞の増殖活性に影響がないことが確認された。以上から，レーザー光照射による細胞傷害がないことが示され，開発手法の安全性を確認した。

次に，組織工学的手法を用いて作製した培養軟骨組織を対象に，開発システムを適用して計測し

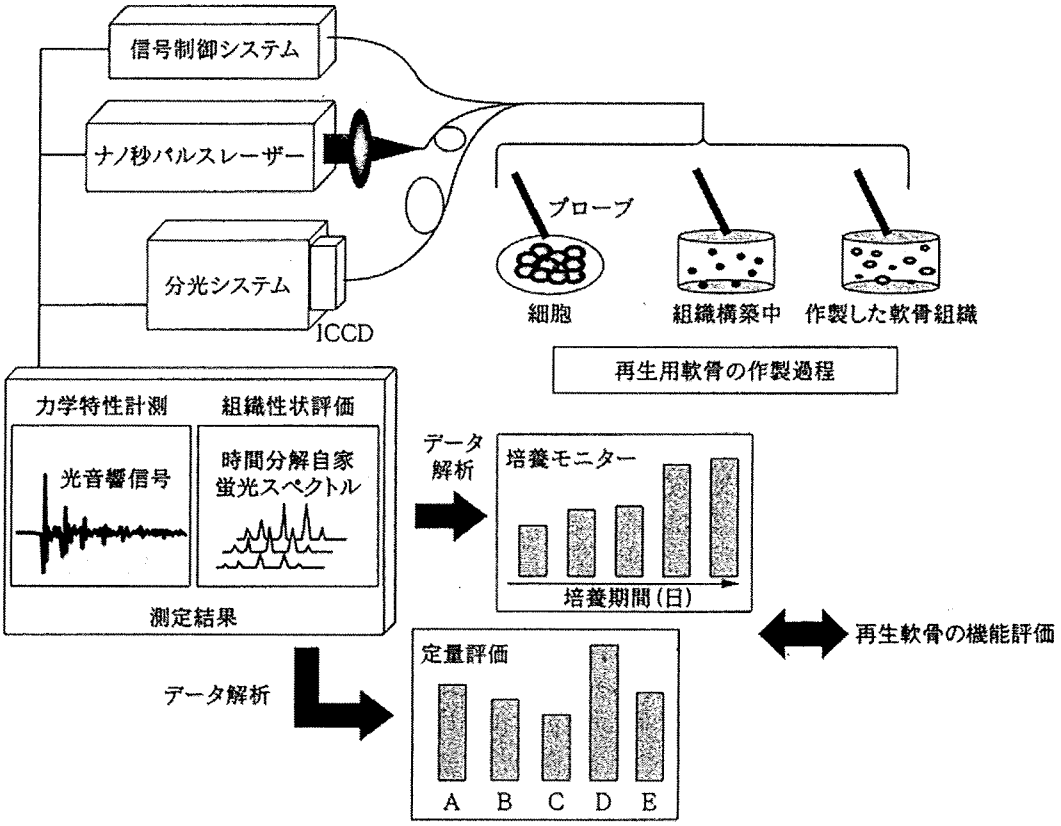


図 1　提案しているシステム[5]

ナノ秒パルスレーザーを光ファイバーで導光し，作製過程の再生用軟骨に，プローブを介して照射して，検出した測定信号から，力学特性計測と組織性状評価を実施する。非侵襲的に測定できるので，培養モニターが可能となり，定量値が算出できるので複数のサンプルの定量評価が可能となる。

た。結果の1例を図3に示す[7]。この実験に用いた培養担体は，共著者の佐藤らが開発した膜付アテロコラーゲンハニカムスポンジ（ACHMS-scaffold）である[8][9]。本担体はアテロコラーゲンからなり，ハニカム状の構造で，大きさは48ウェルプレートに合わせた外径11 mm，厚さ2 mmである。また，細胞保持のために片面がコラーゲン膜でシールドされている。組織工学における培養担体は，細胞を保持し分化誘導する極めて重要な役割を担う。既に本担体を培養担体として椎間板線維輪細胞を培養した実験において，高密度かつ三次元培養が可能であり，軟骨としての形質を維持しながら長期間培養が可能であることが示されている。培養担体に日本白色家兎（体重約1 kg）から膝関節軟骨を摘出して酵素処理（コラゲナーゼとアクチナーゼ処理）により単離した軟骨細胞を高密度（1×10^6 細胞/担体）で播種し，三次元培養を12週間行った。図3に示すように，結果として培養軟骨組織の培養期間をパラメーターとして，減衰時間をプロットした。幼弱家兎の摘出膝関節軟骨を対象にした結果も比較のために示した。培養期間が長くなるにつれて，応力波の減衰時間は短くなり，正常軟骨の値に近づき，培養期間が12週の培養軟骨組織で正常軟骨に比べて測定された力学特性は約88%であった。計測した培養軟骨組織の細胞外マトリックスを生化学的に分析したところ，粘弾性値の経時変化とコラーゲン量，コンドロイチン硫酸量の変化には相関係数0.9以上が得られ，細胞外マトリックスの構築過程を反映した力学特性が得られた。以上より，開

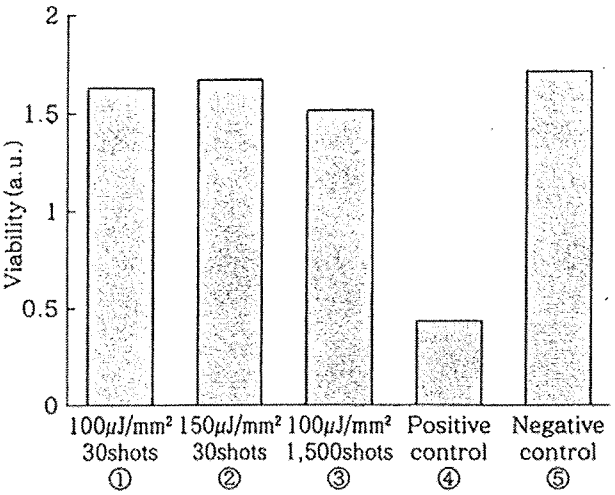
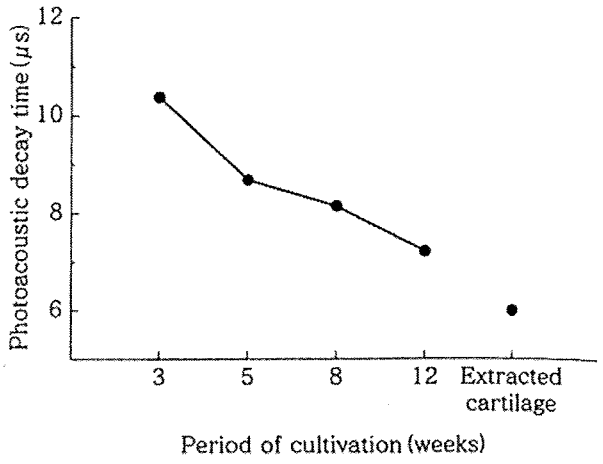


図 2　レーザー光照射による細胞障害試験[6]
①本測定で使用する照射条件，②測定で使用する照射条件よりもパルスエネルギーが 1.5 倍大きい，③測定で使用する照射条件よりもパルス数が 50 倍多い，④細胞が死滅する条件として，アルコールを滴下する群，⑤レーザー光を照射しない群。



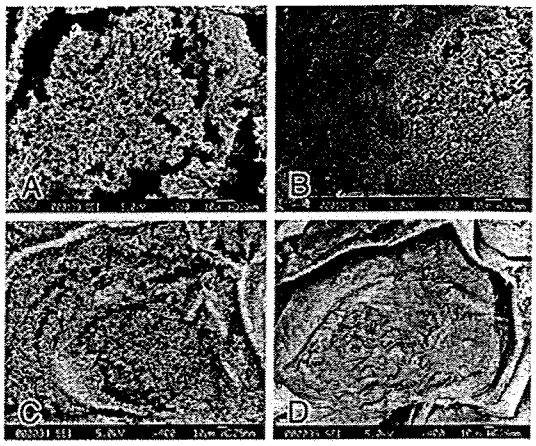


図 3　組織工学的手法を用いて作製した軟骨組織の培養過程において光音響法で経時的に測定した力学特性とその走査型電子顕微鏡像[7]
A　培養 3 週(800 倍)　　B　培養 12 週(800 倍)
C　培養 3 週(400 倍)　　D　培養 12 週(400 倍)

発手法が軟骨再生医療の基盤技術の一つである評価法として有用であることを実証した。

軟骨の再生過程だけでなく，関節軟骨の変性に伴う変化を評価できれば，開発システムが高齢社会で急増する変形性関節症の診断法として適用可能となると着想し，検証実験を既に開始している。酵素を用いた変性モデルを対象にした実験では，変性の進行度によって光音響法で測定される粘弾性パラメーターが変化することを既に明らかにした。2005 年より東海大学臨床研究審査委員会承認のもと，この光音響法を関節鏡視下での軟骨変性診断に適用している。ヒト膝関節軟骨を対象に計測した結果から，組織学的変化と力学特性パラメーターの変化が相関することを確認した。現在測定できる力学特性は，組織の粘性（η）と弾性（G）の比（η/G）に相当する応力波の減衰時間（τ）であるが，粘性（η）と弾性（G）の絶対値を測定できるように，測定に利用するレーザーの波長を複数にするシステム構築の検討を開始している。また，再生軟骨の評価技術として標準化をすることで，軟骨再生医療の早期実用化を目指している。

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