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Dynamic Quasi-Elastic Light Scattering Measurement of Biological Tissue

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Abstract

During laser irradiation, mechanically deformed cartilage undergoes a temperature dependent phase transformation resulting in accelerated stress relaxation. Clinically, laser-assisted cartilage reshaping may be used to recreate the underlying cartilaginous framework in structures such as ear, larynx, trachea, and nose. Therefore, research and identification of the biophysical transformations in cartilage accompanying laser heating are valuable to identify critical laser dosimetry and phase transformation of cartilage for many clinical applications. Quasi-elastic light scattering was investigated using Ho : YAG laser ($\lambda = 2.12\ \mu m$; $t_p \sim 450\ \mu s$) and Nd:YAG Laser ($\lambda = 1.32\ \mu m$; $t_p \sim 700 \mu s$) for heating sources and He : Ne ($\lambda = 632.8$ nm) laser, high-power diode pumped laser ($\lambda = 532$ nm), and Ti : Al_2O_3 femtosecond laser ($\lambda = 850$ nm) for light scattering sources. A spectrometer and infrared radiometric sensor were used to monitor the backscattered light spectrum and transient temperature changes from cartilage following laser irradiation. Analysis of the optical, thermal, and quasi-elastic light scattering properties may indicate internal dynamics of proteoglycan movement within the cartilage framework during laser irradiation.

Key words : biophysical transformation, cartilage, laser, light scattering, macromolecules, phase transition, stress relaxation, tissue

I. INTRODUCTION

Light scattering data may be used to characterize its structures and internal interactions in a material [14-16]. Light scattering techniques have been widely used to investigate phase transitions in macromolecular solutions and solid materials[14-16]. In the study of cartilage, since cartilage has the important functional properties such as stiffness, durability, and distribution of load depending on the extracellular matrix, the light scattering technique has been performed for different applications. Reihanian *et al.* attempted to demonstrate this method for studying hydrodynamic properties of proteoglycan subunit from bovine nasal cartilage[17]. Kovach *et al.* focused on the mechanical properties of cartilage using He : Ne Laser($\lambda = 632.8$ nm)[18]. In the laser-mediated cartilage reshaping technique, first introduced by Sobol using CO_2 laser($\lambda = 10.6\ \mu m$) in the ear and nose of rabbits and human cartilage, light scattering has been used to investigate the phase transformation in cartilage following laser irradiation[5, 6]. Sobol *et al.* presented that under the effect of laser irradiation, mechanically deformed cartilage undergoes a temperature dependent phase transformation resulting in accelerated stress relaxation and hypothesized that cartilage can be affected by the distribution of internal stresses from a local laser heating and thus reshape without ablation [4, 5]. In a later study of laser-mediated cartilage reshaping, Sobol *et al.* measured temperature and stress in cartilage under the irradiation with a Holmium laser and established that the reshaping of cartilage is connected with the bound-to-free transformation of water at a temperature around 70℃[6-9]. On the other hand, Wong *et al.* investigated integrated backscattered light intensity from He : Ne laser and radiometric surface temperature changes of cartilage during laser irradiation using a Nd:YAG laser ($\lambda = 1.32\ \mu m$) [10,13]. Wong *et al.* presented that internal stress and integrated backscattered light intensity were observed to increase, plateau, and then decrease during laser irradiation[10, 13]. Wong *et al.* also attempted to determine the critical temperature transition in laser-mediated cartilage reshaping[11]. Light scattering, infrared radiometry, and modulated differential scanning calorimetry were used for the measurement[11]. From this experiment, Wong demonstrated the simultaneous recordings of integrated backscattered light intensity, internal stress, and average surface temperature. The laser irradiation time was about 9 to 10 seconds for fast heating experiments and the conventional calorimetric measurement time was about 30 minutes for slow heating experiments[10-13]. The principal object of this study is to identify the changes in light scattering of cartilage during

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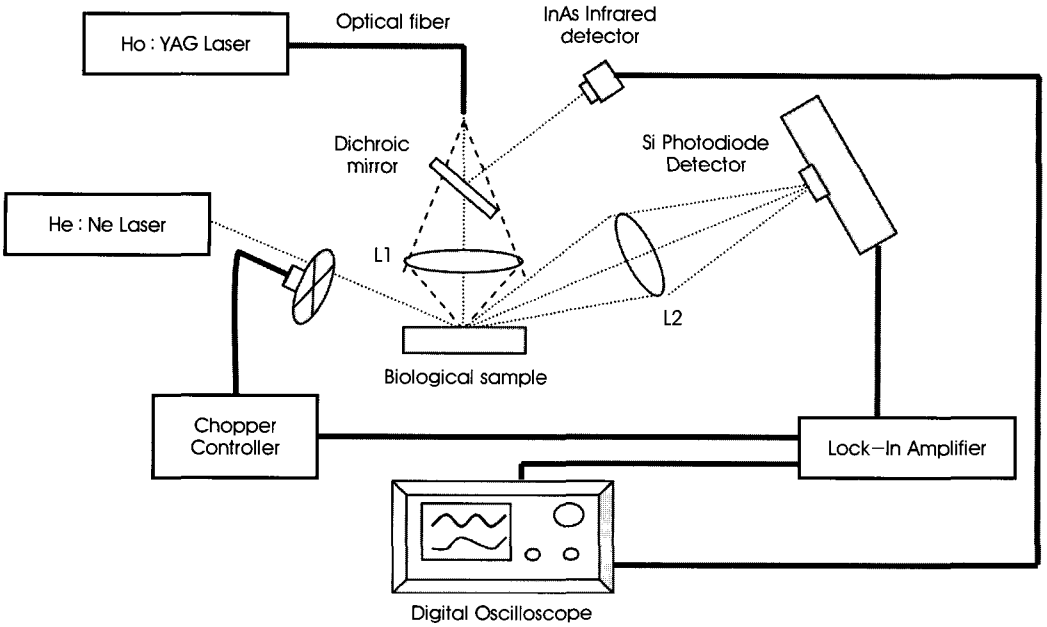

Fig. 1. Schematic layout of dynamic light scattering measurement for fast heating response of cartilage using He:Ne Laser.

short pulsed laser irradiation(~ 500 μs). Quasi-elastic light scattering was investigated using a Ho:YAG laser(λ = 2.12 μm) for a heating source and He:Ne and 532 nm lasers for light scattering sources. Time resolved measurements of changes in light scattering during rapid heating are valuable to identify critical laser dosimetry and phase transformation of nasal septal cartilage for many clinical applications as a non-invasive technique.

II. EXPERIMENTAL METHODS

A. Sample Preparation

The same procedure of the cartilage extraction from a fresh porcine head as described in the previous chapter was performed and the samples were stored in physiological saline. Prior to the dynamic light scattering measurement of nasal septal cartilage, the specimens were cut into rectangular shapes approximately 0.4mm × 0.4mm × 0.15mm using a scalpel. The specimens were then placed a petri dish in physiological saline.

B. Dynamic Light Scattering Measurement of Cartilage using He : Ne Laser

As seen in Figure 1, Ho:YAG laser(λ = 2.12 μm, P = 0.2-2 Watts, VersaPulse 2.1, Model 2000, Coherent Inc.) delivered by a 550μm core-diameter SiO_2 low OH- fiber was applied to heat the cartilage specimens in Fig. 1.

The Ho:YAG laser beam reflected from a glass to an indium arsenide infrared detector(model J12-18C-R250U, EG&G Optoelectronics Inc.) to display a Ho:YAG pulse signal and focused on a cartilage specimen by lens 1(L1). He:Ne laser(λ= 632.8 nm, 0.5 mW, Novette, Uniphase Inc.) light was modulated(3 kHz) with a mechanical chopper using a controller (Model SR540, Stanford Research System Inc.). The chopper controller and silicon PIN photodetector(Model 2031, New Focus Inc.) were connected to a lock-in amplifier(Model LIA 100, ThorLabs Inc.) to detect a dynamic light scattering signal of cartilage. The two laser beams were aligned to overlap each other and set up for the same beam size. The Ho:YAG laser pulse and He:Ne laser light scattering signal were synchronously detected by the indium arsenide infrared detector and the silicon photodiode detector, respectively and displayed by a digital oscilloscope (Model TDS 640A, Tektronics, Inc.). The data were acquired by each pulse of the Ho:YAG laser at 2.0 J with the spot size of 3 mm(~28.2 J/cm^2). The spot size of the Ho:YAG laser was estimated using a thermal burn paper and the laser power was measured with a laser/energy power meter(Model EPM2000e, Molectron Inc.).

C. Dynamic Light Scattering Measurement of Cartilage using 532nm Laser

As seen in Fig. 2, a 532 nm laser (Millennia, high-power diode pumped laser, Spectra-Physics Laser Corp.) was used instead of a He:Ne Laser to examine light scattering at an alternative wavelength.

A polarizer was used to the 532 nm laser beam for the

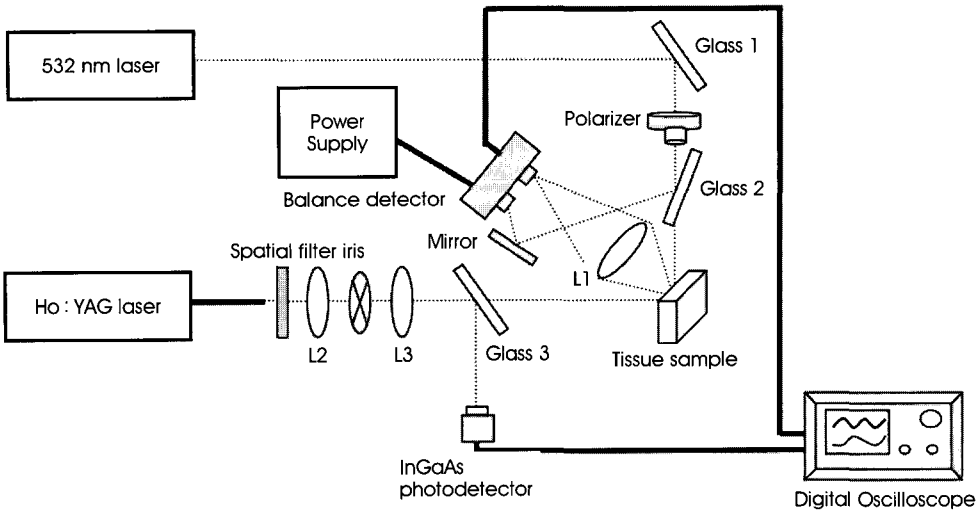

Fig. 2. Light Scattering Measurement for fast heating response of cartilage using 532 nm Laser.

optimal light signal backscattered by a biological sample. The 532 nm diode-pumped laser beam was split into reference and sample beams by glass 2. The reference beam reflected from mirror and focused on the reference arm of a auto-balanced photodetector (Model 2007, 125KHz, New Focus Inc.). The other traveled through a cartilage specimen with the spot size of 3 mm was scattered and focused into the signal arm of the balanced detector. The Ho:YAG laser beam traveled trough an optical attenuator to eliminate light from the flash-lamp of the laser. The beam reflected from glass 3 was then focused into the window of the Indium Gallium Arsenide photodetector (Model SU75-2.2-TO, Sensors Unlimited, Inc.) to display Ho:YAG laser signal and also focused on a cartilage specimen. The specimen size was similar to the previous experimental setup and positioned on a circluar microslide on top of the post

III. RESULTS

A. Dynamic Light Scattering Signal of Cartilage Using He : Ne Laser

Dynamic light scattering signal of He:Ne laser was acquired simultaneously corresponding to the single pulse of the Ho: YAG laser in Figure 3. The scattered light signal from cartilage started to increase immediately after the single pulse of the Ho:YAG laser was applied to the specimen and rapidly decreased as a function of time following laser irradiation. The laser irradiation was for approximately 0.5 ms and the peak of the light scattering signal occurred prior to the cessation of laser radiation.

B. Dynamic Light Scattering Signal of Cartilage Using 532nm Laser

Figure 4 depicts the phase transformation of cartilage using 532 nm laser following the laser irradiation of the Ho:YAG laser. The intensity of the Ho:YAG laser was applied to four different ranges such as 28.2, 25.4, 22.5 and 11.3 J/cm^2(Fig. 4). The light scattering signal from 532 nm laser was not seen below 11.3 J/cm^2. The fractional change in light scattering intensity for cartilage was increased following the laser radiation and reached a peak prior to the cessation of single pulse of the Ho:YAG laser, then subsequently decreased.

The laser irradiation lasted approximately 0.5 msec and the light scattering signal was dependent upon the intensity of the laser radiation. The higher intensity of Ho:YAG laser radiation applied, the more light scattering occurred. These findings were consistent with the previous experimental setup.

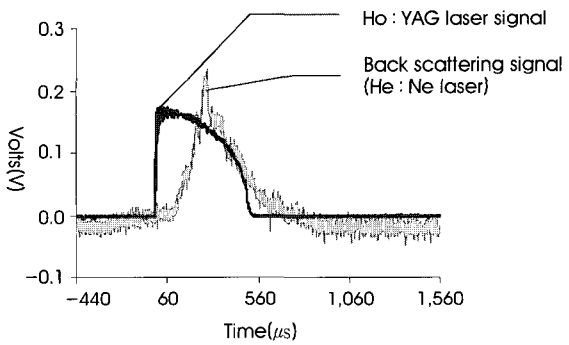

Fig. 3. Fast response of cartilage irradiance using a He : Ne laser(λ= 632 nm) for a light scattering source and 28.2 J/cm^2 of a Ho:YAG laser for a heating source.

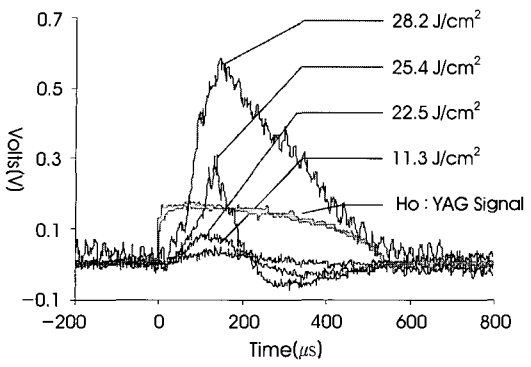

Fig. 4. Fast response of cartilage irradiance using 532 nm laser for a light scattering source at different power of Ho : YAG laser.

Ⅳ. DISCUSSION

As demonstrated by the results, the stress relaxation of cartilage may depend on the laser wavelength, pulse duration, irradiance, and energy intensity of laser source. For other quasi-elastic light scattering studies in laser-assisted cartilage reshaping technique, Sobol *et al* presented the typical behavior of the light scattering signal in cartilage under CO_2 laser irradiation for 10 seconds with the laser intensity of 50-70 W/cm^2 [7]. Wong *et al* also observed the internal stress and fractional change in the integrated backscattered light intensity for 9.5 seconds as the laser irradiation time with Nd:YAG laser power of 38.45 W/cm^2[8]. For fast laser heating response in our study, although different laser sources and laser irradiation time were applied, the changes of backscattered light signal from cartilage in Figures 3 and 4 were similar to the slow heating response measured by Sobol *et al* [4-9] and Wong *et al* [10-13].

One consideration for fast laser heating response technique is the elimination of the noise sources. Since the acquisition time was relatively short, small changes in backscattered light signal, and the noise from ambient lighting and instrumentation interfered with the signal acquisition during the experiment. To overcome this limitation, various lock-in detection methods and experimental setup should be applied to enhance the backscattered light signal. An additional consideration is about the temperature measurement for fast heating response of cartilage. Because the laser irradiation time is only for 500 sec, it was difficult to detect the radiometric temperature changes in cartilage during laser irradiation. Nevertheless, since the radiometric signal contains information concerning the surface temperature of cartilage, the real-time measurement of tissue temperature should be performed to modulate laser energy intensity using a relatively fast response infrared detector.

Sobol *et al* suggested that the changes of light scattering in cartilage may be due to the isolation of water movement, leading to an increase of backscattered light intensity. Furthermore, they assumed the molecular basis for thermal mediated stress relaxation may be due to the nucleation of water droplets in the course of a phase transition, although the molecular basis of thermal mediated stress relaxation is not known [7]. Alternatively, Wong *et al* assumed that the process of relaxation may be partially due to the denaturation of collagen or proteoglycan [10]. If heating is sustained for a long time at above 65℃ on tissue, proteins begin to undergo denaturation and unwind their helix [10]. Inasmuch as protein denaturation is dependent upon time and temperature, Wong *et al* recommended that rapid laser heating may be advantageous to reshape cartilage without the denaturation or ablation[10]. To identify detailed internal dynamics of substitutes within cartilage following laser irradiation, an additional experiment should be performed using isolated macromolecule (i.e. collagen, proteoglycan) solution. This measurement may provide significant data to identify important molecular events in laser-assisted cartilage reshaping. Since Sobol *et al* and Wong *et al* irradiated cartilage relatively for a long period of time (~10 sec) and high intensity, more dehydration of cartilage and loses its elastic properties may occur than our study as a result of laser treatment. As a consequence, the fast laser heating technique (i.e. ~500 sec) for cartilage reshaping may be superior to the slow heating technique (i.e. ~10 sec) to minimize nonspecific thermal injury and chondrocyte death. There is another consideration in this experimental approach that requires further experiments to clarify. Since the light scattering is the angular dependence as the result of the product of structure factor and the scatter pattern from individual fibers within cartilage, various experimental setups regarding the scattered light angle may be significant interpretation in terms of structure and arrangement of macromolecules within the cartilaginous framework [18]. Furthermore, although the exact laser parameters are unknown, various laser sources and parameters should be performed to minimize nonspecific tissue injury and preserve chondrocytes to be viable.

Ⅴ. CONCLUSIONS

The results of this study demonstrate a relationship between the dependence of energy intensity from heating source and the phase transformation of cartilage following laser irradiation. Furthermore, the backscattered light pattern from cartilage may contain detailed information about the spatial arrangement and internal dynamics of macromolecules (e.g. collagen, proteoglycan) during laser irradiation.

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