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Effect of wavelength on threshold and kinetics of tissue denaturation under laser radiation

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ABSTRACT

We consider the denaturation process as an alteration in ordered organization of tissue structure and study the threshold and kinetics of laser-induced denaturation in cartilage and cornea undergoing irradiation from a free electron laser (FEL) in the wavelength range 2.2–8.5 μ. Light-scattering by cartilage samples was measured in real-time during FEL irradiation using a 630-nm diode laser and a diode array with time resolution of 10 ms. We found that denaturation threshold is slightly lower than that for cartilage, and both depend on laser wavelength. A strong inverse correlation between denaturation thresholds and the absorption spectrum of the tissue is observed. Only for the wavelength region near the 3 μ water absorption band was the denaturation threshold not inversely proportional to the absorption coefficient. We believe this was because the radiation penetration depth was very small in this high-absorption region, so tissue denaturation occurred only in a layer too thin to produce significant light scattering. ATR spectra of 2.4 mm thick cartilage samples was measured before and after irradiation at 6.0 and 2.2 μ. At 6.0 μ, where the absorption is high, the spectrum of the irradiated (front) surface showed changes, while the spectrum of the back surface was identical to that before irradiation. This difference results from dramatic denaturation (with chemical bond breaking) at the front surface due to laser heating in a small absorption depth. For 2.2 μ irradiation, where the absorption is small, the spectra of the front and back of the irradiated sample were unchanged from before irradiation, wile light scattering alteration shown the denaturation process began, for laser fluences above the denaturation threshold. This indicates that the absorption is too small to produce deep denaturation of the tissue with dramatic alteration of structure. Thus, we have shown that light scattering is useful for measuring denaturation thresholds and kinetics for biotissues except where the initial absorptivity is very high.

Keywords: Denaturation, light scattering, absorption spectra, cartilage, cornea

1. INTRODUCTION

Tissue denaturation by IR lasers is a thermally and mechanically mediated process which sets an upper boundary for low-energy procedures, such as reshaping of cartilage, and determines the thermal damage for high-energy laser processes, such as tissue ablation and welding[1]. Laser-induced alteration of tissue structure changes the number and size of light-scattering centers in the tissue. It allows us to consider the denaturation as an alteration in ordered organization of tissue native structure and to study the process by means of optical techniques. Cartilage is a well suited biopolymer to use the light scattering technique because a characteristic size of proteoglycan units (hundreds of nm) and aggregates (1 μ) are near the wavelength of visible light[2].

Laser light scattering technique has been widely used for structural characterization of proteoglycans in solutions[3-5] and for laser-induced stress relaxation in cartilage[6,7]. A strong correlation between light scattering signal and mechanical properties of cartilage was documented also in the ref. 8. To the present the data on correlation between light scattering and rapid structure alterations in real biotissues under pulse laser radiation are very limited, and to our knowledge the effect of laser wavelength on denaturation processes is not studied in details jet.

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The aim of this paper is to study the wavelength dependencies of threshold and kinetics of denaturation in cartilage and cornea undergoing irradiation from a free electron laser in the wavelength range 2.2–8.5 μ.

2. MATERIALS AND METHODS

Fresh pig nasal septums and eyes were used to prepare samples of hyaline cartilage and cornea. Separated nasal septums and eyes were placed between gauze sponges soaked in 9% saline and stored at 2-4 °C in a closed box. The samples for investigation were prepared immediately before experiment by cutting out disks of 9.8 mm in diameter with a thickness of 1.3 mm for cartilage, of 0.9 mm for pig eyes cornea. The samples were fastened in a special holder allowing to irradiate the area of 6 mm in diameter. Every sample was used only for one irradiation procedure. Also 8 cartilage samples (2.4 x 5 x 25 mm) were prepared for FTIR spectra measurements.

The scheme of experimental set-up is presented in Fig.1. The Free Electron Laser (FEL) at the Vanderbilt University (Nashville, Tennessee) was used for samples irradiation in the wavelength range from 2.2 to 8.5 μ. The temporal structure of a superpulse of FEL beam is a train of pulses of 1 ps in duration following in 350 ps each from another during about 6 mks[9]. The typical energy of a superpulse was 20-30 mJ, the pulse repetition rate was of 20 Hz. The following wavelengths were used for tissue irradiation: 2.2, 2.5, 2.81, 3.0, 3.22, 3.5, 3.9, 4.68, 5.3, 6.0, 6.1, 6.45, 6.7, 6.85, 7.2, 7.4, 7.8, 8.05, and 8.5 μ (Fig.2). The FEL beam passed through germanium Brewster separator which reflects $1/n$ harmonics and transmits FEL radiation. FEL beam was focused on a sample surface using a lens of 272 mm focal length to provide necessity power density. The Frenel attenuator consisting from the number of CaF_2 plates was also used to control the FEL power density. All samples irradiated were examined with a surgical microscope Zeiss, Universal S3 (Germany) with magnification x60 to manifest any visible alterations of the surface.

The main measured parameters in this study is the kinetic of light intensity distribution of probe beam on the front surface of the sample being irradiated. The light of a diode laser of 600-700 wavelength band width and of about 5mm in diameter was delivered from back side of a sample and using a lens with 190 mm focal length focused on its front surface to a spot of about 1mm in diameter.

The image of the front surface was transferred on the plane of a photodiode array with a lens of 80 mm focal length with magnification x 2,8. Such procedure provides measurement of the light intensity distribution on the front surface of the sample. Optical system was aligned to measure the intensity distribution along horizontal plane oversecting the center of FEL beam spot on the sample.

Photodiode array included 3700 elements located along a line with 8mkm distance between neighboring elements. The light intensity distribution was registered using a Multichannel Optical Registration System and the software produced by the MORS Limited, Russia. The computer card was operated in the regime of external triggering. The triggering pulse was generated simultaneously with the opening of a shutter. This started up the recording of a series of light intensity distribution on the plane of photodiode array in the frame series mode. Every frame was exposed from 50 to 250 ms. Typical recording time was 20 seconds. Thus 80 files with data of light distribution after each sample irradiation were usually saved for the subsequent mathematical processing. Attenuated total reflectance (ATR) FTIR spectroscopy was used to examine infrared tissue absorption spectra before and after irradiation. Several samples irradiated with the FEL at 6.0 μ (when the absorption coefficient is high) and 2.2 μ (with small absorptivity) were examined with a spectrometer Bruker IFS 66V. Tissue was irradiated with the 2.5 mm in diameter laser spot at the laser fluence of about 6 J/cm^2 for 15 s. Than the FEL beam was moved on 2.5 mm along the central line of the 5 x 25 mm sample surface, and the next spot was irradiated, and so on from point to point.

3. RESULTS AND DISCUSSION

Cartilage and cornea have shown very similar absorption spectra (Fig.2). Both these tissues consist from water, collagen and proteoglycans, but have different structures. Some differences in spectra represent mainly to the variations in water content (up to 70 percent, for cornea and up to 80 percent, for cartilage). This makes these tissue interesting for comparative examination of laser-induced structural alterations. using the light scattering technique. Photographs of cartilage and cornea irradiated are shown on Fig. 3. From light scattering distribution measured for different instances we have derived the light scattering kinetics as time dependencies of the maximal light intensity (I_m),

half width (w), and intensity distribution integral (A). Some of these dependencies are plotted on the Figure 4 for different laser wavelengths and fluences.

It is seen from the data that light distribution becomes brooder and the intensity of light go through a sample decreases with time and laser fluence. For low laser fluence, neither alterations in light distribution were seen during the period of observation nor visible changes on the surface irradiated were observed using a light microscope. For higher fluence, visible alterations in I_m, w, and A start at some instances depending on laser fluence and wavelengths. When laser fluence is high enough (0.29 J/cm^2, for λ = 5.30 μ, and 0.38 J/cm^2, for λ = 8.05 μ) the prolonged irradiation results in increase of I_m. For these samples, we have seen a luminescence during irradiation and also a hollow and some darkening on the surface irradiated. We believe that above observations manifest tissue carbonization followed by ablation resulting in decrease of optical density of the sample.
From the plots shown on the Fig.4 (and from other similar data) we can obtain the denaturation delay t_* as the instance when A drops up to 10 percent from its initial value.

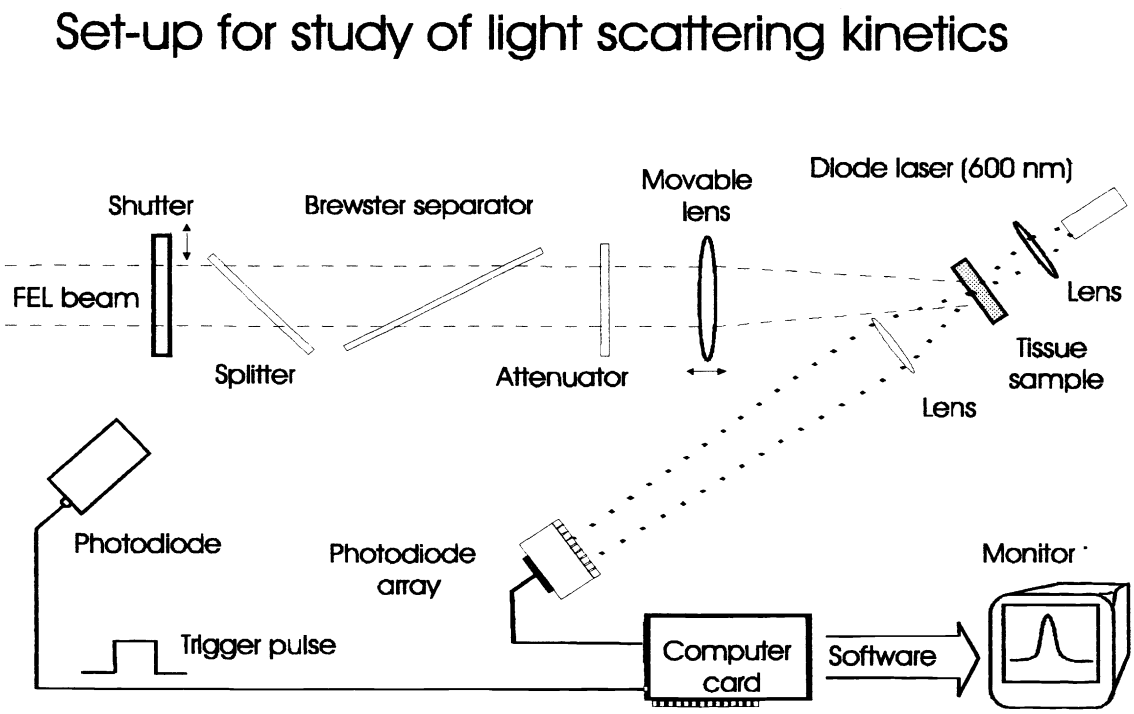

Figure 1. Set-Up for real time light scattering monitoring of cartilage denaturation under laser radiation

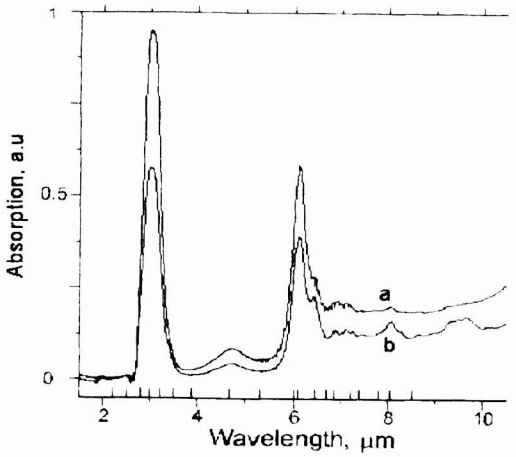

Figure 2. ATR FTIR spectra of (a) cartilage and (b) cornea and the wavelengths examined

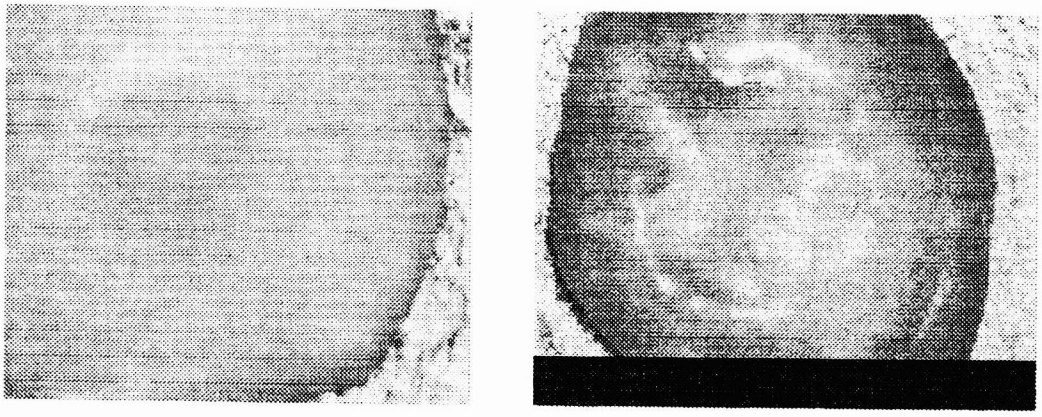

Figure 3. (a) Cartilage and (b) cornea samples irradiated.

Wavelength dependency of denaturation threshold is shown on the Figure 5. An inverse correlation between denaturation thresholds and the absorption spectrum of the tissue is observed. Only for the wavelength regions near the 3 and 6 μ water absorption bands was the denaturation threshold not inversely proportional to the absorption coefficient. We believe this was because the radiation penetration depth was very small in this high-absorption region, so tissue denaturation occurred only in a layer too thin to produce significant light scattering. Similar reason could be in charge of some peculiarities in kinetics of denaturation. Approximation of denaturation kinetics with a function

$$A = A_u + (1 - A_u) \exp(-kt) \qquad (1)$$

allows us to find kinetic coefficient k as the functions of laser wavelength and fluence. The formulae (1) can be derived from the first order of phase transformation kinetics[1] $dA/dt = -kA$. under an assumption that the integral of light intensity go through a sample is proportional to the amount of non denatured tissue. This rough description allows us to characterize the denaturation kinetics with only one kinetic coefficient k and to study the effect of laser fluence and wavelength on k. More detailed analysis of denaturation kinetics is presented in 10. A values of k drop to zero with decreasing in F. This is another way to get the denaturation threshold fluences which are differ (but no more than on 30 percent) from the values F_{th} found from the dependencies t^* on F.

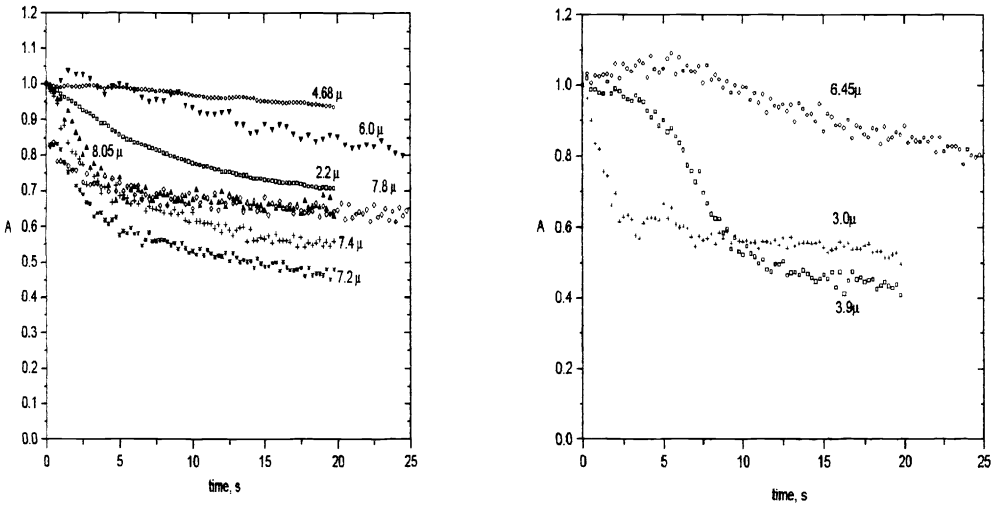

Figure 4. Light scattering kinetics for different laser wavelengths at F ≅ 0.1 J/cm^2
(a) cartilage, (b) cornea

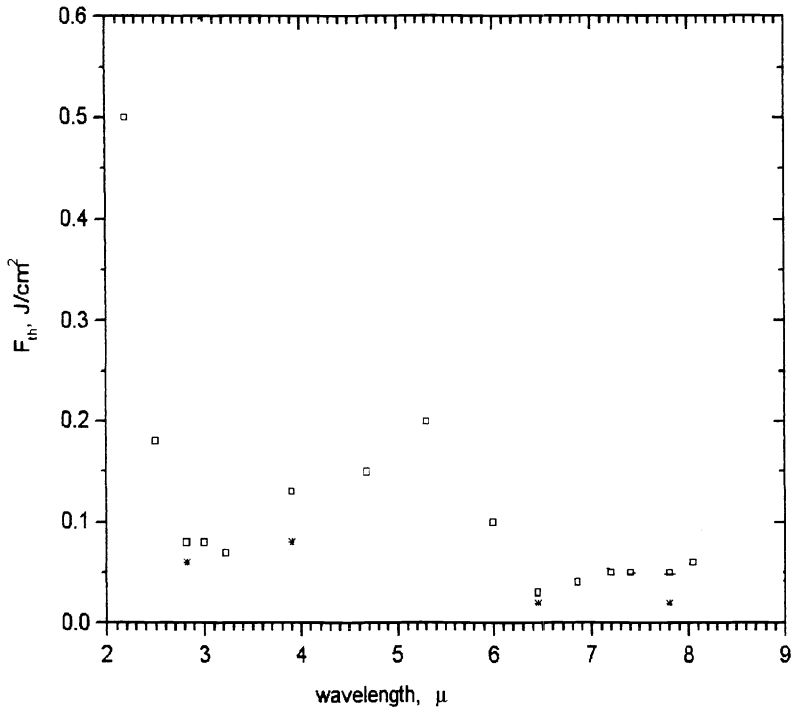

Figure 5. Wavelength dependency of denaturation threshold
(□)cartilage, (*) cornea

The results obtained allow to find the optimal laser wavelengths when it is necessary to accelerate or to impede the denaturation process. It is also followed from these data that k values generally increase with light absorption coefficient of a tissue, but it is not always true. For example, k is higher for 6.45 μ than that for 6.0 μ It is opposite to the relation between the absorption coefficients for above wavelengths. Perhaps it could be due to heterogeneous absorption of light by different component of cartilage. The specific absorption of collagen for 6.45μ and its effect on laser-tissue interaction was previously established and discussed in the Ref. 11. We do not know the reasons of some increasing in k for wavelengths near 8 μ. From the other hand, in this region, the absorption coefficients is less than that in high absorption bands near 3 and 6 μ, and higher than that for other wavelengths studied. We believe that the dependency of k on α may have a maximum because, for small absorption coefficients, denaturation goes slowly as the temperature is not high enough; for high α, the radiation penetration depth is small, so tissue denaturation occur only in a layer too thin to be detected with light scattering technique. This assumption is in agreement with results of spectroscopic examination. Figure 6 presents the ATR spectra of a cartilage sample measured before and after irradiation. At 6.0 μ, where the absorption is high, the spectrum of the irradiated (front) surface showed changes, while the spectrum of the back surface was identical to that before irradiation. This difference results from dramatic denaturation (with chemical bond breaking) at the front surface due to laser heating in a small absorption depth. At 6.0 μ, we overheated the irradiated surface to produce significant light scattering. As the sample was thick enough to heat the back surface, the spectrum of the back surface was identical to that before irradiation. For 2.2 μ irradiation, where the absorption is small, the spectra of the front and back of the irradiated sample were unchanged from before irradiation, although light scattering shows that denaturation ha begun. This indicates that the absorption is too small

to produce deep denaturation of the tissue with dramatic alteration of structure. So, the light scattering technique is more sensitive that spectroscopic technique to study the beginning of denaturation process when laser energy is higher (but not very much) than the threshold of denaturation.

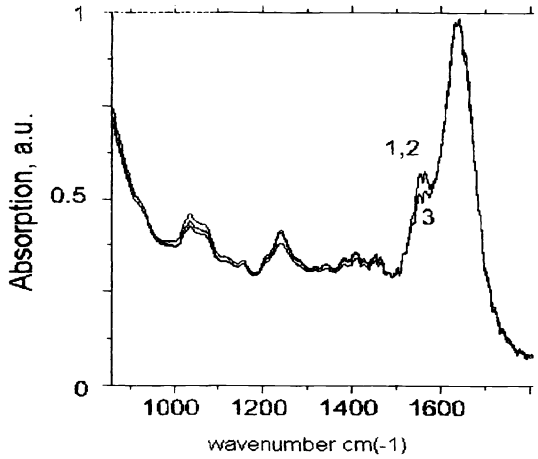

Figure 6 ATF FTIR spectra of cartilage: (1) non-radiated sample, (2) back surface of the irradiated sample, (3) front surface of the irradiated sample. $\lambda = 6.00\ \mu$

4. CONCLUSIONS

Effect of laser wavelength on energy threshold and kinetic coefficients for cartilage and cornea denaturation was studied for the first time. We found that since cartilage and cornea have very similar absorption spectra, the denaturation threshold for cornea is slightly lower than that for cartilage. An inverse correlation between denaturation thresholds and the absorption spectrum of the tissue is observed. Only for the wavelength regions near the 3 and 6 μ water absorption bands was the denaturation threshold not inversely proportional to the absorption coefficient. Light scattering is more sensitive technique than ATR FTIR for measuring denaturation thresholds and kinetics for biotissues at IR region except wavelengths near 3 and 6 μ where the initial absorptivity is very high.

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