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Free Electron Laser Ablation of Articular and Fibro-Cartilage at 2.79, 2.9, 6.1, and 6.45 μm: Mass Removal Studies

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Background and Objective: The wavelength and tissue-composition dependence of cartilage ablation was examined using selected mid-infrared laser wavelengths.
Study Design/Materials and Methods: The mass removal produced by pulsed laser ablation of articular and fibro-cartilage (meniscus) were measured. The wavelengths examined were 2.79, 2.9, 6.1, and 6.45 μm and provided by a free electron laser (FEL) emitting 4 μsecond macropulses consisting of 1–2 picoseconds duration micropulses delivered at 350 picosecond intervals. The measurement of tissue mass removal was conducted using a microbalance during laser ablation.
Results: These studies demonstrated that for articular cartilage the highest mass removal was achieved at $\lambda =$ 6.1 μm followed by, in order, $\lambda = 2.79$, 2.9, and 6.45 μm. In comparison, the maximum mass removal for fibro-cartilage was achieved using $\lambda = 6.1$ μm radiation with no statistically significant differences in mass removal provided by the other wavelengths. In evaluation of the comparative influence of each wavelength on tissue type, there was no difference in ablation efficiency between articular and fibro-cartilage at $\lambda = 6.1$ μm. However, the ablation efficiency of articular cartilage was higher than that of fibro-cartilage at both $\lambda = 2.79$ and 2.9 μm. By contrast, $\lambda = 6.45$ μm radiation ablated fibro-cartilage more efficiently than articular cartilage at radiant exposures greater than 12 J/cm^2.
Conclusions: The mass removal of articular and fibro-cartilage produced by FEL ablation at selected mid-IR wavelengths was measured as a function of incident radiant exposure. The ablation efficiency was found to depend on both wavelength and tissue type. The 6.1 μm wavelength was found to provide the highest ablation efficiency for both articular and fibro-cartilage. Lasers Surg. Med. 36:202–209, 2005. © 2005 Wiley-Liss, Inc.

Key words: ablation; ablation efficiency; articular cartilage; fibro-cartilage; free electron laser; laser; meniscus; tissue mass loss

INTRODUCTION

Lasers have been evaluated for the effective cutting or ablation of intraarticular tissue for arthroscopic surgery. Several studies have evaluated the effect of various laser parameters including wavelength (λ), pulse duration (τ), pulse repetition rate (f), and incident radiant exposure (Φ_0) on the ablation of cartilage [1–18]. Previous studies suggest that the 2.9 μm wavelength, absorbed predominantly by the water OH-stretch mode, results in an explosive vaporization event and is most efficient for cartilage ablation [4,18]. However, several studies have indicated that wavelengths absorbed by the tissue matrix instead of, or in addition to, the tissue water may prove advantageous in increasing mass removal and reducing residual damage [19–22].

Tissue optical properties, tissue composition, and optical penetration depth are all important factors affecting ablation dynamics and residual tissue injury. Water and protein are the principal chromophores in cartilage in the infrared (IR) spectral region. The absorption peaks of water are located at $\lambda = 0.96$, 1.44, 1.95, 2.94, 4.68, 6.1 μm; the largest of which are situated at 2.94 and 6.1 μm [23]. The effects of the dynamic optical properties of water when irradiated by Er:YSGG ($\lambda = 2.79$ μm) and Er:YAG ($\lambda =$ 2.94 μm) laser radiation have been analyzed and have demonstrated that 2.79 μm radiation offers better spatial confinement of the deposited laser energy than 2.94 μm radiation for incident radiant exposures exceeding 0.5 J/cm^2 [24]. Primary absorption peaks of protein in the IR are located at 6.1, 6.45, and 7.87 μm and are governed by amide I, II, and III absorption bands, respectively [25]. At 6.1 μm, the absorption by collagen is two times larger than that of water while at 6.45 μm the absorption of collagen is roughly six times larger than water [25].

The free-electron laser (FEL) is a unique biomedical research tool due to its broad wavelength tunability and temporal pulse structure. Edwards et al. [19] first demonstrated remarkable properties of 6.45 μm radiation to

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ablate ocular, neural, and dermal tissues with little collateral damage. This study stated that the direct targeting of protein using $\lambda = 6.45$ μm provides tissue removal through protein modification, rather than by explosive vaporization resulting from the heating of water [19]. Experimental investigation with cortical bone has also been performed with the FEL to compare collateral thermal damage as a function of wavelength [22]. Peavy et al. [22] found that the use of wavelengths in the 6.1–6.45 μm region were the most efficient for cutting cortical bone with minimal collateral thermal damage. On the other hand, other studies indicate that 6.1 but not 6.45 μm radiation provides both optimal ablation efficiency and reduced tissue thermal injury [26–31]. An investigation of corneal ablation using FEL wavelengths between 2.94 and 6.7 μm by Bende et al. [26] found that minimal collateral damage was achieved at 6.0, 6.1, and 6.3 μm but not 6.45 μm. Fowler et al. [30] found the least collateral damage is produced for corneal ablation at 6.1 μm (amide I stretch). Heya et al. and Jean and Bende [27,31] also demonstrated that the gelatin was efficiently ablated by FEL at the wavelength of 6.1, but not 6.45 μm. Thus, the effect of tissue type on the selection of wavelength to achieve efficient FEL ablation with minimal residual injury is not fully resolved or understood. In this study, in order to examine the effect of tissue chromophore (water vs. protein) on ablation efficiency, we examine this process at the selected wavelengths, 2.79, 2.9, 6.1, and 6.45 μm. Moreover, the examination of both articular and fibro-cartilage ablation allows for an examination of the effect of tissue composition as articular cartilage contains 70% water and 30% protein while fibro-cartilage contains 55% water and 45% protein [32]. Both 2.79 and 2.9 μm were chosen because of the reported temperature dependence in optical absorption of water in the 3 μm region [24]. In addition, 6.1 μm was chosen to examine cartilage ablation when targeting protein and water simultaneously while 6.45 μm was chosen to examine ablation by targeting primarily tissue protein.

MATERIALS AND METHODS

Fresh bovine cadaver knees were obtained from a local abattoir. The patella, distal femur, proximal tibia, and both menisci were isolated following removal of supporting muscle, tendons, ligaments, and joint capsule. The shaft of the femur was transected just above the patellar groove. The distal femur, patella, and mensci were individually wrapped in 0.9% saline soaked gauze sponges and cotton toweling, placed in sealed plastic bags and stored at 4°C until used. Just prior to each experiment a specimen bag was removed from refrigeration and allowed to equilibrate to room temperature. Individual specimens were unwrapped as needed, and a 4 mm biopsy punch was used to harvest cartilage samples for laser irradiation. Specimens remained covered in saline soaked gauze between each sample collection. Following ablation each sample was placed in a sealed container of 10% phosphate buffered formalin and refrigerated for future examination.

The experimental setup is shown in Figure 1. A mid-IR FEL tuned to the one of the four wavelengths of interest,

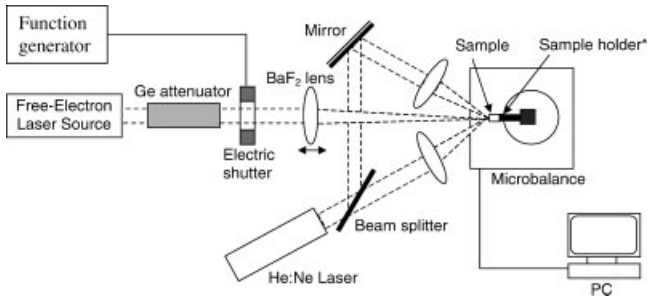

Fig. 1. Schematic of experimental setup for mass loss measurements.

2.79, 2.9, 6.1, or 6.45 μm, was used to ablate the cartilage specimens. The FEL delievers 4.0 μsecond macropulses composed of a series of 1–2 picoseconds micropulses delivered 350 picoseconds apart. Depending on the pulse energy the macropulse repetition rate was set to 5 or 10 Hz, resulting in the delivery of 50 or 100 pulses over the fixed exposure time of 10 seconds. Separate measurements demonstrated no difference of the mass removal at macropulse repetition rates of 5, 10, and 20 Hz. The macropulse energy delivered to the tissue was set to predetermined values between 10 and 40 mJ/pulse, and confirmed using an energy meter (Model EM500, Molectron, Inc., Portland, OR). Five cartilage samples were ablated for the mass loss measurements at each pulse energy tested.

The FEL beam was directed through a custom made germanium attenuator and an electronic shutter (Model 04IES 211, Melles Griot) connected to a function generator (Model 33120A, Agilent Tech, Rochester, NY) for control of the exposure time. The collimated FEL beam was directed through a BaF_2 lens with a focal length of 200 mm into a 500 μm diameter spot at the tissue surface. By adjusting the BaF_2 lens on a translational stage, the location along the focused beam at which the beam diameter was 500 ± 10 μm was determined for each wavelength by use of a custom designed motorized knife-edge instrument. A pair of diode laser ($\lambda = 635$ nm, Model S1FC635, Thorlabs, Inc., Newton, NJ) beams were aligned to intersect at this predetermined focal position of the FEL beam and were used to locate the target plane for precise placement of each cartilage specimen. Each cartilage specimen was placed in a 4 mm diameter biopsy punch mounted on the pan of the digital balance (resolution = 10 μg, Model AG 183, Mettler Toledo, Inc., Columbus, OH) as depicted in Figure 2 so that any

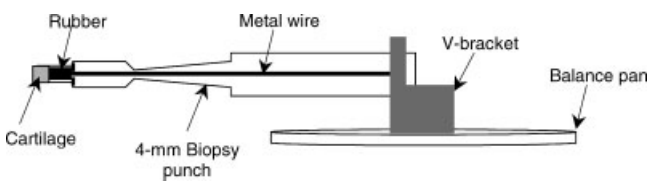

Fig. 2. Schematic of cartilage sample holder.

ablated material would not land on the pan. The digital balance was connected through a serial port to a personal computer. The tissue mass loss was recorded in real-time at a rate of 5 Hz and stored in a hard disc for future analysis.

Tissue mass loss from laser ablation was calculated by the difference between the total mass loss and the mass loss attributable to water evaporation. Since the mass loss measurements require correction for tissue dehydration by evaporation, linear regression was applied to the slope of mass loss after laser irradiation to determine the "corrected mass loss" as shown in Figure 3 [33,34].

RESULTS

FEL ablation of cartilage was quantified by measuring the mass of tissue removed (μg/pulse) with a microbalance during laser ablation. Figure 4 shows the measured mass loss of articular and fibro-cartilage versus incident radiant exposure at each wavelength. The mass removal of articular cartilage was higher than that of fibro-cartilage at both $\lambda = 2.79$ and 2.9 μm (Fig. 4A,B). However, there was no clear tissue type dependence for articular and fibro-cartilage ablation at $\lambda = 6.1$ μm (Fig. 4C). By contrast, $\lambda = 6.45$ μm radiation was more effective in removing fibro-cartilage than articular cartilage at radiant exposures greater than 12 J/cm^2 (Fig. 4D).

Figure 5 plots ablation efficiency (μg/J) versus radiant exposure for both articular and fibro-cartilage ablation at each wavelength. Ablation efficiency, η_{abl}, is defined as the mass of tissue removed per unit energy delivered to the tissue and given by $\eta_{abl} = M/\Phi_0 A$, where M is the mass of tissue removed (μg), Φ_0 is the incident radiant exposure at the tissue surface (J/cm^2) and A is the irradiated area (cm^2) [24]. At $\lambda = 2.79$ and 2.9 μm (Fig. 5A,B), ablation of articular cartilage was more efficient than that of fibro-cartilage, while the reverse is true for 6.45 μm ablation at radiant exposures greater than 12 J/cm^2 (Fig. 5D). There was no significant difference in the ablation efficiency between articular and fibro-cartilage at $\lambda = 6.1$ μm (Fig. 5C).

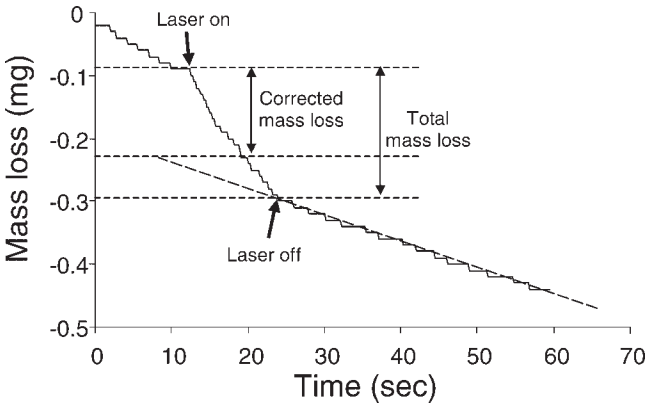

Fig. 3. Tissue mass versus time. The "corrected mass loss" term represents only the laser-induced loss of tissue mass corrected for concurrent environmental dehydration of the tissue specimen.

DISCUSSION

Water and protein are the principal chromophores in cartilage and the water and protein content differ significantly between cartilage types. The results show clear differences in measured mass removal and ablation efficiency are produced by changes in wavelength and tissue composition. At $\lambda = 2.79$ and 2.9 μm, where water is the dominant chromophore, the ablation efficiency of articular cartilage was higher than that of fibro-cartilage which has a lower water content. By contrast, the ablation efficiency of fibro-cartilage, which contains more protein, was higher than that of articular cartilage at $\lambda = 6.45$ μm; a wavelength where protein absorption is dominant. No clear difference in ablation efficiency was observed in articular and fibro-cartilage at $\lambda = 6.1$ μm; a wavelength where both protein and water are important chromophores.

The wavelength dependence of mass removal versus incident radiant exposure for the four wavelengths is given for articular and fibro-cartilage in Figure 6A, B, respectively. Error bars are omitted for clarity. For articular cartilage mass removal is greatest at $\lambda = 6.1$ μm and the least at $\lambda = 6.45$ μm. This result is in contrast to the observation of Edwards et al. who observed larger mass removal of cornea, which consists of 80% water and 20% protein, at 6.45 μm when compared to 6.1 μm [19,35]. A t-test for each wavelength at radiant exposures between 10 and 15 J/cm^2 confirms a statistically significant difference in mass removal between all the wavelengths ($P < 0.05$). In the water dominant absorption region at $\lambda = 2.79$ and 2.9 μm, the ablation of articular cartilage at 2.79 μm is higher than at 2.9 μm ($P < 0.1$). For fibro-cartilage, the maximum mass removal was provided by 6.1 μm wavelength ($P < 0.001$), and there were no statistically significant differences in mass removal between the other three wavelengths.

Ablation efficiency (μg/J) as a function of incident radiant exposure at the four wavelengths tested is shown for articular and fibro-cartilage in Figure 7A, B, respectively. Ablation efficiency for both cartilage types using $\lambda = 6.1$ and 6.45 μm is roughly constant within the range of radiant exposures from 5 to 20 J/cm^2, while ablation efficiency of articular cartilage at both $\lambda = 2.79$ and 2.9 μm approach that of $\lambda = 6.1$ μm at radiant exposures larger than 15 J/cm^2. Based on the data illustrated in Figures 6 and 7, the 6.1 μm wavelength, where both water and protein possess absorption peaks, is the most efficient wavelength for the ablation of both articular and fibro-cartilage. This result is consistent with the findings of both Heya et al. and Jean and Bende [27,31] in that 6.1 μm ablation is more efficient than 2.79/2.9 μm ablation where water is the sole absorber. In this fashion, this result is also consistent with the study of Payne et al. who found that the TEA CO_2 laser ablation of skin at 9.5 μm, where both water and protein are both important chromophores, is more efficient than ablation at 10.6 μm where water is the sole absorber [36].

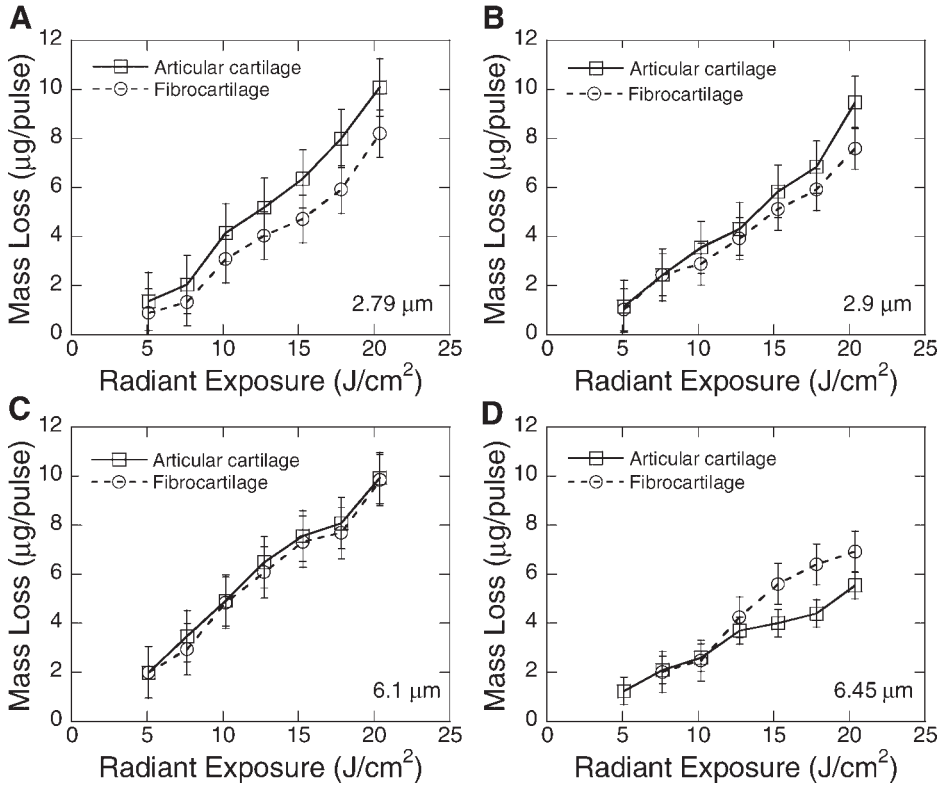

Fig. 4. Comparison of articular and fibro-cartilage mass removal versus incident radiant exposure at four different wavelengths; 2.79 μm (**A**), 2.9 μm (**B**), 6.1 μm (**C**), and 6.45 μm (**D**).

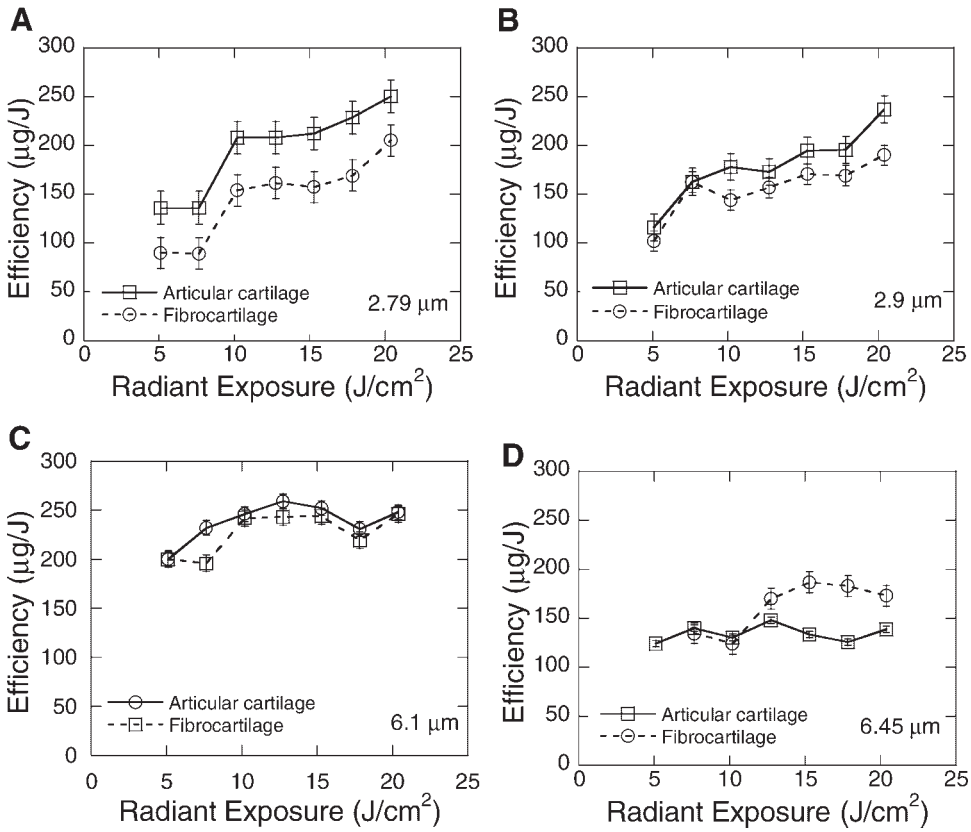

Fig. 5. Comparison of articular and fibro-cartilage ablation efficiency versus incident radiant exposure at four different wavelengths; 2.79 μm (**A**), 2.9 μm (**B**), 6.1 μm (**C**), and 6.45 μm (**D**).

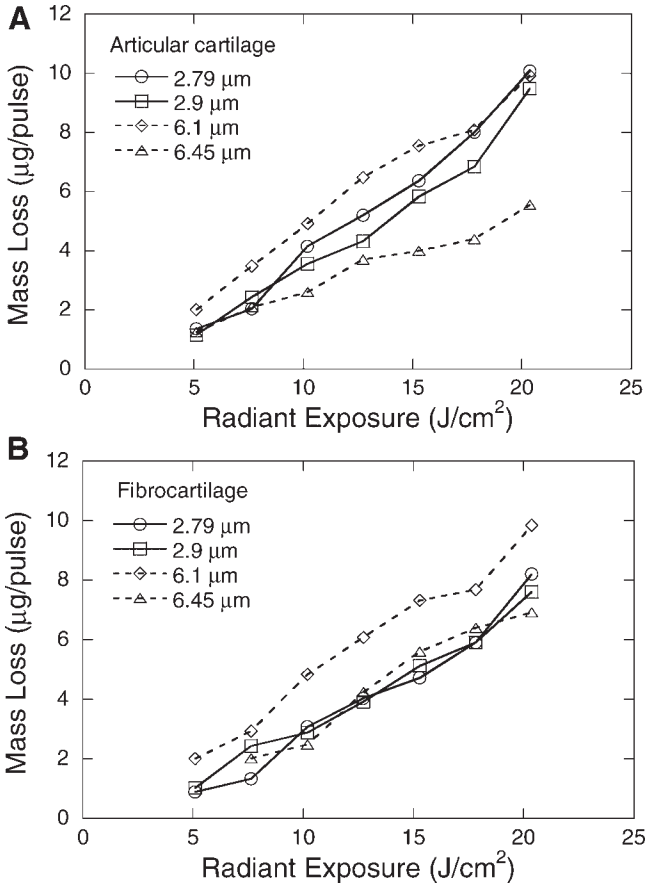

Fig. 6. Mass removal versus incident radiant exposure for articular (**A**) and fibro-cartilage (**B**) for four different wavelengths. The error bars are not shown for clarity.

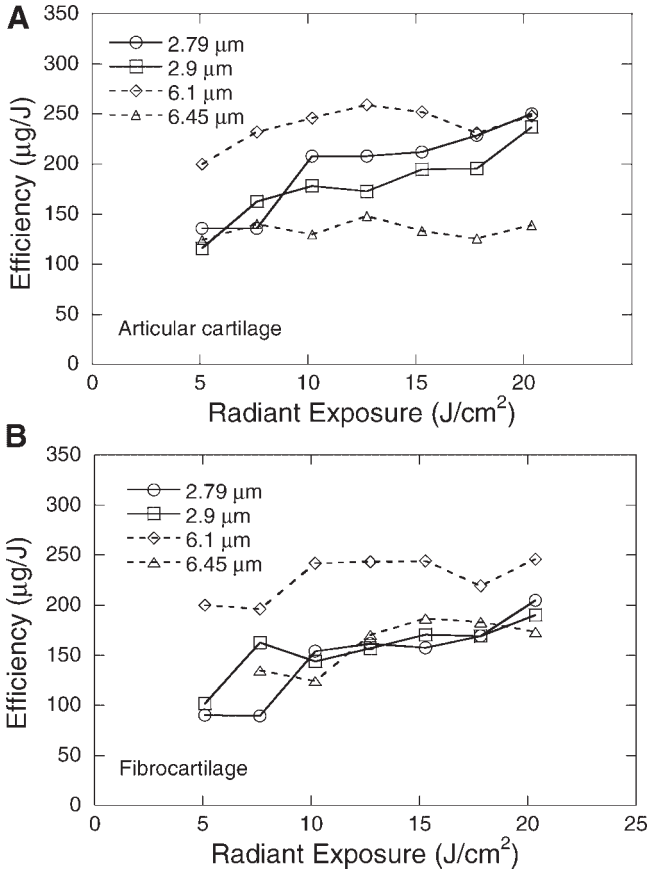

Fig. 7. Ablation efficiency versus incident radiant exposure for articular (**A**) and fibro-cartilage (**B**) for four different wavelengths. The error bars are not shown for clarity.

The amount of tissue removal is dependent upon the total amount of energy delivered to the tissue and tissue ablation can be quantified by measuring the tissue mass loss as a function of pulse energy by two first-order models: (1) steady-state model and (2) blow-off model [24,33]. In our case, the steady-state model is more appropriate because material is removed during the laser pulse [33]. The steady-state model provides a linear relationship between the tissue mass loss and the radiant exposure given by $M = A(\Phi_0 - \Phi_{th})/h_{abl}$ for $\Phi_0 > \Phi_{th}$ where A is the area radiated, and h_{abl} is the ablation enthalpy (J/g) that defines the energy required to ablate a unit mass of tissue [24,33]. The steady-state model is applied to our mass removal data and plotted for both articular and fibro-cartilage in Figure 8A, B, respectively. The ablation enthalpy was calculated for each wavelength and tissue type from the slope of the mass loss versus radiant exposure curve and presented in Table 1. The measured ablation enthalpy of articular and fibro-cartilage can be compared to the heat of vaporization of water in room temperature (~2,500 J/g) and the activation energy for chain scission or depolymerization of tissue molecules (~1,200 J/g) [19,34]. Generally, if the heat of ablation is less than 2,500 J/g, tissue removal does not occur solely by vaporization, but by the explosive removal of tissue or recoil-induced material removal [24]. In our study, the ablation enthalpy for both articular and fibro-cartilage was greater than 2,500 J/g. This is likely due to the fact that when using μsecond long laser pulses, there is significant shielding of the incident radiation by the ejected tissue debris [24,37].

To examine possible correlations between the optical absorption properties and the absorption characteristics, we calculated absorption coefficients (μ_a) and optical penetration depth of each tissue using the absorption properties for water and collagen in proportion to their presence in each tissue (Table 2) [22]. In the 3 μm region, where water is the dominant absorber, the ablation enthalpy of fibro-cartilage is higher than that of articular cartilage. In the 6.1 μm region, there was no clear difference in ablation enthalpies of both articular and fibro-cartilage. However, the ablation enthalpy for articular cartilage is almost 60% higher than that of

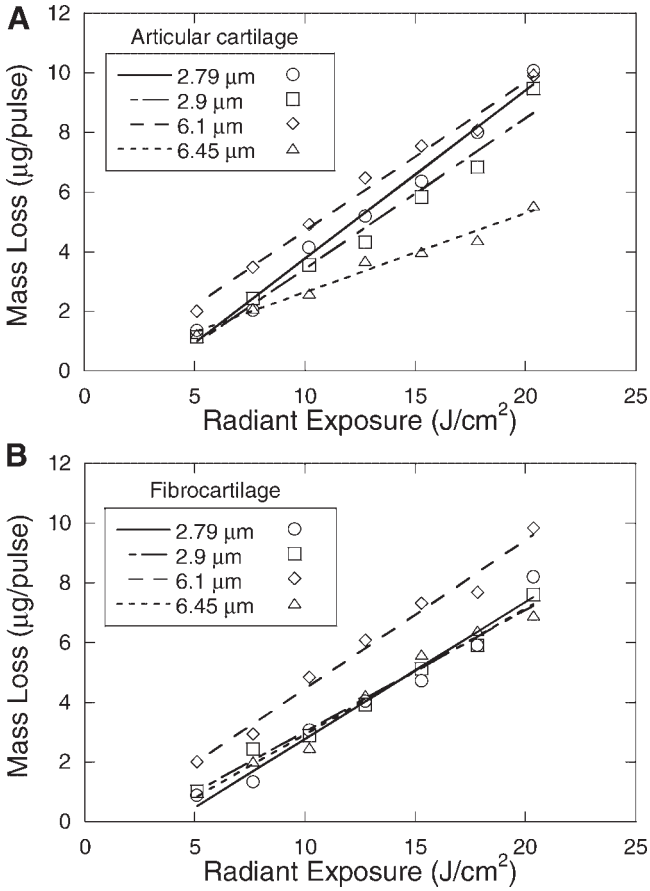

Fig. 8. Linear regression model plots of articular (**A**) and fibro-cartilage (**B**). The solid lines are the calculated mass removal versus radiant exposure based upon the linear regression model and the markers are the experimental mass loss data from Figure 6.

fibro-cartilage at $\lambda = 6.45$ μm. The ablation efficiencies of articular and fibro-cartilage at 15 J/cm^2 versus the optical penetration depth ($1/\mu_a$) of collagen and water are plotted in Figure 9A, B, respectively. For fibro-cartilage, there is a clear, monotonically decreasing relationship between the ablation efficiency and the optical penetration depth of collagen suggesting that high absorption by collagen results in high ablation efficiency. However, no correlation was found for articular cartilage (Fig. 9A). In Figure 9B we see that for articular cartilage there is a gradual increase in ablation efficiency with increasing optical penetration depth of water with the exception of the outlying data point generated by 6.45 μm. The reduction in ablation efficiency with increasing water absorption may result from an increased water vapor content of the ablation plume thereby shielding a larger fraction of the delivered laser energy and cause the observed reduction of the ablation efficiency [37]. No correlation was found on optical penetration depth of water and ablation efficiency in fibro-cartilage (Fig. 9B).

TABLE 1. Ablation Enthalpy of Articular and Fibro-Cartilage

	λ (μm)	Articular cartilage	Fibro-cartilage
Ablation enthalpy (J/g)	2.79	3,475	4,280
	2.9	3,885	4,838
	6.1	3,932	3,946
	6.45	7,396	4,653

CONCLUSION

This study has shown that both articular and fibro-cartilage are most efficiently ablated when using the 6.1 μm wavelength. In the 3 μm region, the articular cartilage ablation at $\lambda = 2.79$ μm was more efficient than at $\lambda = 2.9$ μm and approaches the efficiencies of 6.1 μm ablation of articular cartilage. However, there was no statistical difference in the characteristics of these two wavelengths for the ablation of fibro-cartilage. We observed the lowest ablation efficiency of articular cartilage at $\lambda = 6.45$ μm, but no statistical difference was observed for fibro-cartilage ablation at $\lambda = 2.79$, 2.9, and 6.45 μm. Collectively, these results demonstrate a distinct advantage in using laser wavelengths that target both water and protein components of biological tissues with respect to improving mass removal and ablation efficiency.

The ablation enthalpy of fibro-cartilage in the 3 μm region, where water is the dominant absorber, was higher than that of articular cartilage. This result demonstrates that more energy is required to ablate fibro-cartilage which contains more collagen than articular cartilage. However, at $\lambda = 6.45$ μm where protein is the dominant absorber, more energy is required to ablate articular cartilage than fibro-cartilage. We also found that high absorption by collagen results in high ablation efficiency in fibro-cartilage based the relationship between the ablation efficiency and the optical penetration depth of collagen. However, the gradual increase in ablation efficiency with increasing optical penetration depth of water may be due to an increased water vapor content of the ablation plume thereby shielding a larger fraction of the delivered laser energy. Further investigation is needed to compare the mass removal and ablation efficiency observations to histologic evaluation of collateral thermal injury and ablation surface effects. While these studies have revealed important effects of laser wavelength and tissue chromophore on the mass removal produced by pulsed laser ablation, it is clear that further detailed experimental study and modeling efforts will be required to fully understand these results from a mechanistic level.

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The authors wish to thank William Gabella and John Kozub of the W.M. Keck Free-Electron Laser Center at

TABLE 2. Absorption Coefficients and Optical Penetration Depths ($1/\mu_a$) of Cartilage at Different Wavelengths

λ (μm)	μ_a water (/mm)	μ_a collagen (/mm)	μ_a articular cartilage (/mm)	μ_a fibro-cartilage (/mm)
2.79	516 (1.94)	16 (62.50)	366.0 (2.73)	291.0 (3.44)
2.9	1,161 (0.86)	122 (8.20)	849.3 (1.18)	693.5 (1.44)
6.1	270 (3.70)	848 (1.18)	443.4 (2.26)	530.1 (1.87)
6.45	82 (12.20)	362 (2.76)	166.0 (6.02)	208.0 (4.81)

Articular cartilage: 70% water and 30% protein; fibro-cartilage: 55% water and 45% protein [32]. Numbers in parentheses are the optical penetration depth (μm).

Vanderbilt University and Marie Wilson of the Beckman Laser Institute and Medical Clinic at the University of California, Irvine, for their technical assistance. The US Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation. The views and conclusions contained herein are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the Air Force Research Laboratory or the US Government.

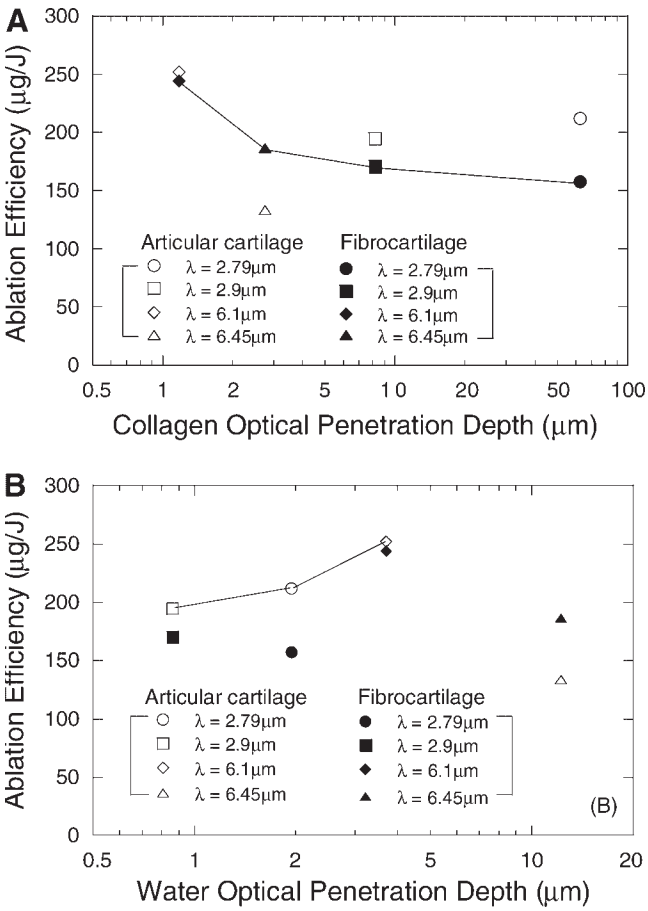

Fig. 9. Ablation efficiency versus optical penetration depth of collagen (**A**) and water (**B**) at the corresponding wavelengths, 2.79, 2.9, 6.1, and 6.45 μm.

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