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Cartilage Ablation Studies using Mid-IR Free Electron Laser

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ABSTRACT

The ablation rate of articular cartilage and fibrocartilage (meniscus), were quantified to examine wavelength and tissue-composition dependence of ablation efficiency for selected mid-infrared wavelengths. The wavelengths tested were 2.9 μm (water dominant absorption), 6.1 (protein and water absorption) and 6.45 μm (protein dominant absorption) generated by the Free Electron Laser (FEL) at Vanderbilt University. The measurement of tissue mass removal using a microbalance during laser ablation was conducted to determine the ablation rates of cartilage. The technique can be accurate over methods such as profilometer and histology sectioning where tissue surface and the crater morphology may be affected by tissue processing. The ablation efficiency was found to be dependent upon the wavelength. Both articular cartilage and meniscus (fibrocartilage) ablations at 6.1 μm were more efficient than those at the other wavelengths evaluated. We observed the lowest ablation efficiency of both types of cartilage with the 6.45 μm wavelength, possibly due to the reduction in water absorption at this wavelength in comparison to the other wavelengths that were evaluated.

Keywords: ablation, ablation efficiency, articular cartilage, fibrocartilage, free electron laser, laser, meniscus, tissue mass loss

1. 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 λ = 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]. 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 [24, 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 [24, 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.* first demonstrated remarkable properties of 6.45 μm radiation to ablate ocular, neural, and dermal tissues with little collateral damage [19]. This study stated that the direct targeting of protein using λ = 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

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the FEL to compare collateral thermal damage as a function of wavelength [22]. Peavy *et al.* found that the use of wavelengths in the 6.1 to 6.45 μm region were the most efficient for cutting cortical bone with less collateral thermal damage [22]. On the other hand, other studies indicate that 6.1 μm 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.* found that minimal collateral damage was achieved at 6.0, 6.1, and 6.3 μm but not 6.45 μm [26]. Fowler *et al.* found the least collateral damage for corneal ablation at 6.1 μm (amide I stretch) [30]. Heya *et al.* and Jean and Bende also demonstrated that the gelatin was efficiently ablated by FEL at the wavelength of 6.1 μm, but not 6.45 μm [27, 31]. 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, 2.9, 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 fibrocartilage contains 55% water and 45% protein [32].

2. 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^{o}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.

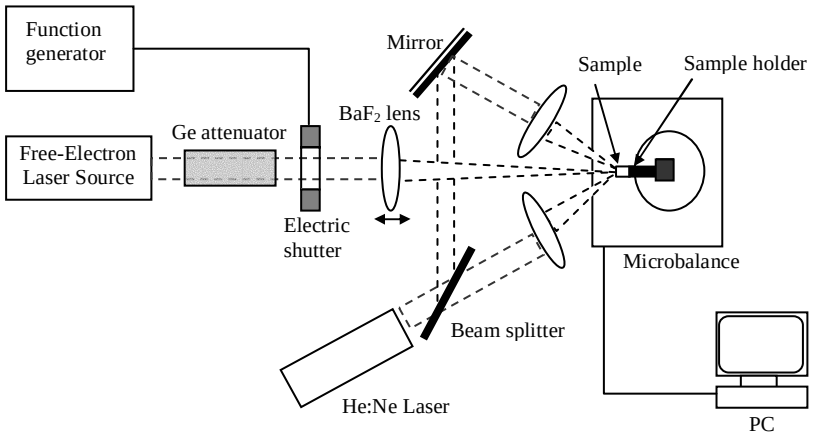

Figure 1. Schematic of experimental setup for mass loss measurements.

The experimental setup is shown in Fig 1. A mid-IR FEL tuned to the one of the three wavelengths of interest, 2.9, 6.1, or 6.45 μm, was used to ablate the cartilage specimens. The FEL delievers 4.0 μs macropulses composed of a series of 1 to 2-ps micropulses delivered 350 ps 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 10mJ/pulse and 40 mJ/pulse, and confirmed using an energy meter (Model EM500, Molectron Inc.). 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.) 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 μm ± 10 μm was determined for each wavelength by use of a custom designed motorized knife-edge instrument. A pair of diode laser (λ = 635 nm, Model S1FC635, Thorlabs Inc.) 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.) so that any 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 2 [33,34].

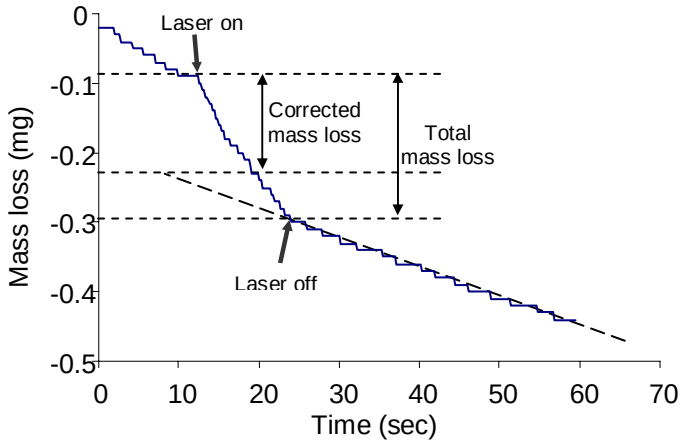

Figure 2. Tissue mass versus time. The 'corrected mass loss' term represents only the laser-induced loss of tissue mass corrected for concurrent environmental dehydration of the tissue specimen.

3. RESULTS AND DISCUSSION

FEL ablation of cartilage was quantified by measuring the mass of tissue removed (μg/pulse) with a microbalance during laser ablation. Figure 3 shows the measured mass loss and ablation efficiency (μg/J) of articular and fibro-cartilage versus incident radiant exposure 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]. The mass removal of articular cartilage was higher than that of fibro-cartilage at 2.9 μm (Figs. 3A and 3B). However, there was no clear tissue type dependence for articular and fibro-cartilage ablation at λ = 6.1 μm (Figs. 3C and 3D). By contrast, λ = 6.45 μm radiation was more effective in removing fibrocartilage than articular cartilage at radiant exposures greater than 12 J/cm^2 (Figs. 3E and 3F).

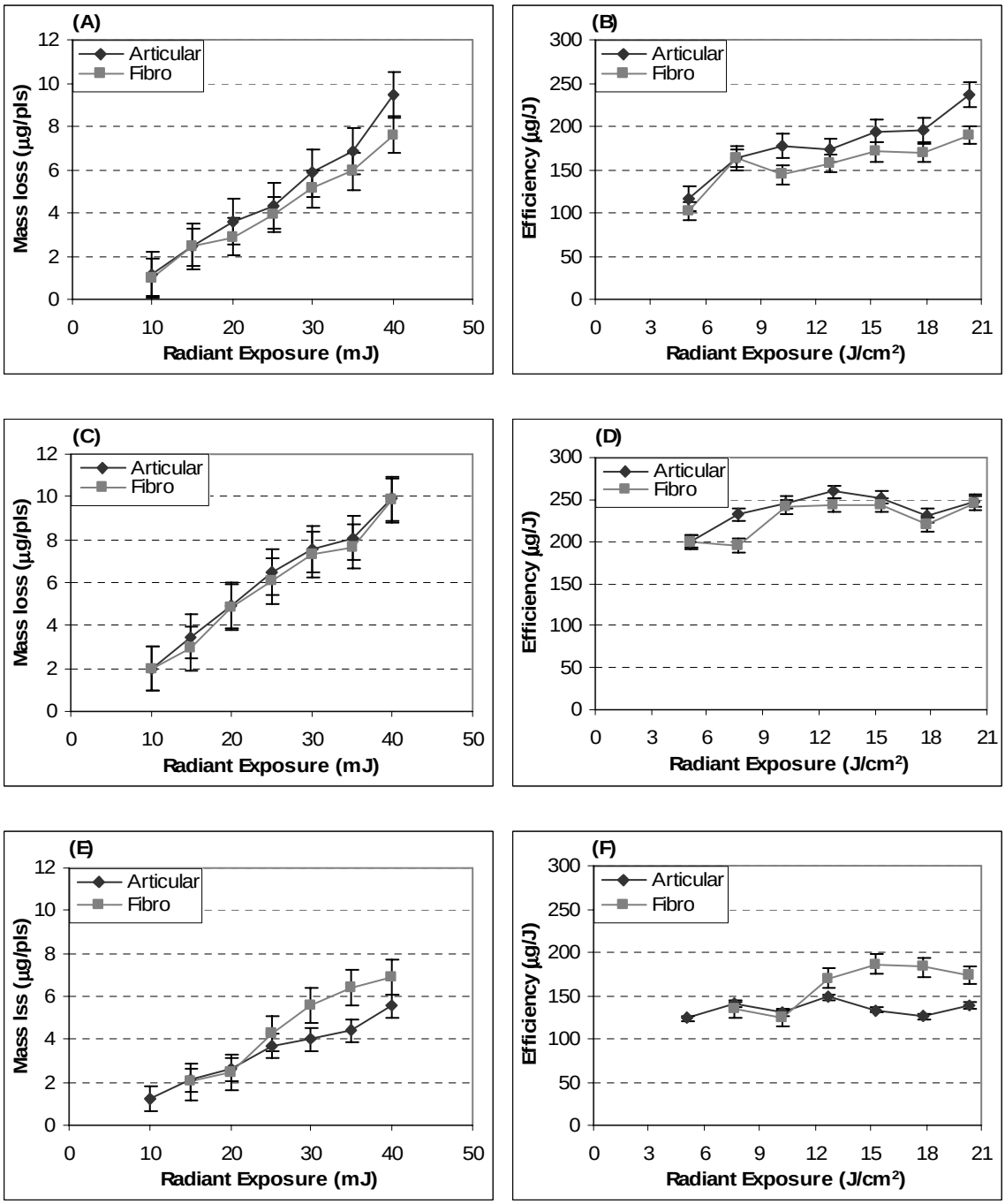

Figure 3. Comparison of articular and fibro-cartilage mass removal and ablation efficiency vs. incident radiant exposure at three different wavelengths; 2.9 µm (A, B), 6.1 µm (C, D), and 6.45 µm (E, F).

Water and protein are the principal chromophores in cartilage and the water and protein content differ significantly between cartilage types. The results show that there are clear differences in measured mass removal and ablation efficiency on both wavelength and tissue composition. At $\lambda = 2.9$ µm, where water is the dominant chromophore, the ablation efficiency of articular cartilage was higher than that of fibrocartilage which has a lower water content. By contrast, the ablation efficiency of fibrocartilage, which contains more protein, was higher than that of articular

cartilage at λ = 6.45 μm; a wavelength where protein absorption is dominant. No clear difference in ablation efficiency was observed in articular and fibro-cartilage at λ = 6.1 μm; a wavelength where both protein and water are important chromophores.

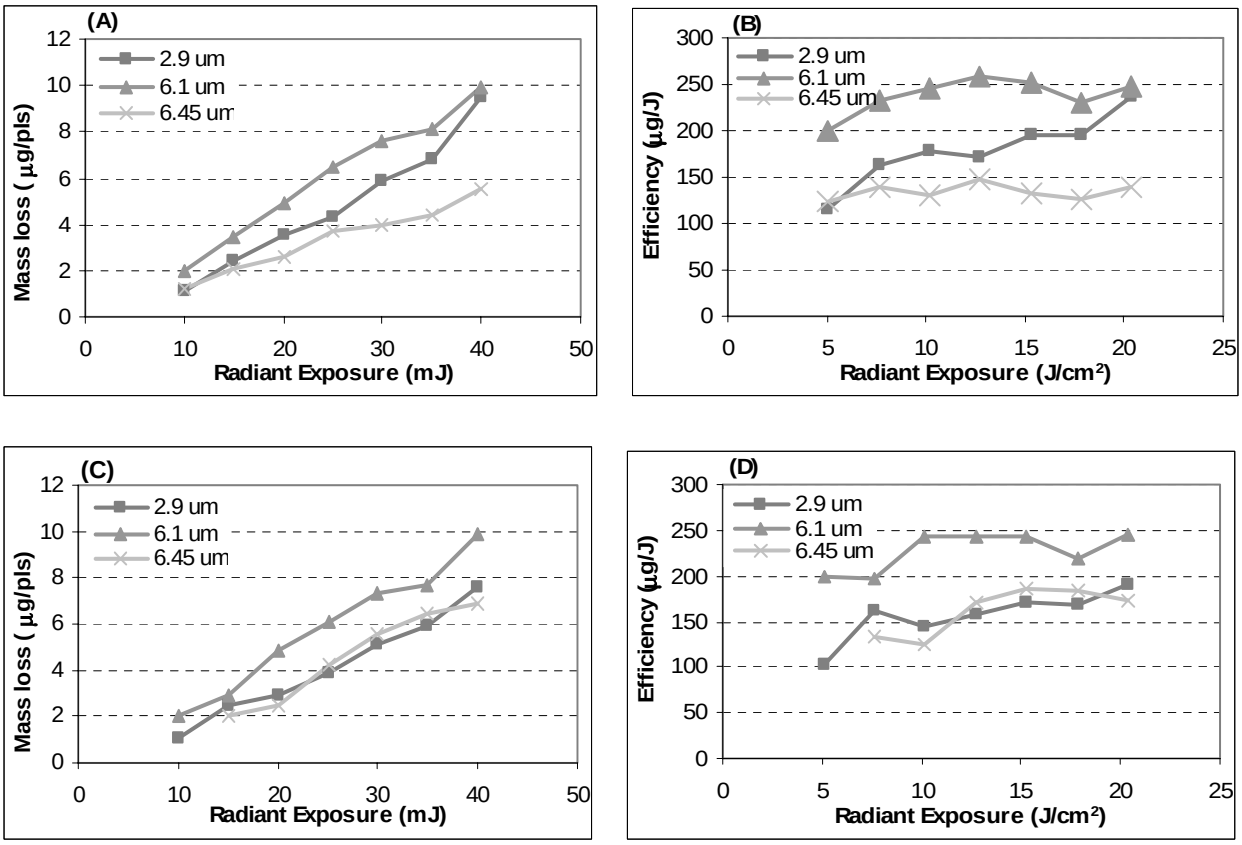

Figure 4. Mass removal and ablation efficiency vs. incident radiant exposure for articular (A, B) and fibro-cartilage (C, D) for three different wavelengths. The error bars are not shown for clarity.

The wavelength dependence of mass removal and ablation efficiency versus incident radiant exposure for the three wavelengths is given for articular and fibro-cartilage in Figures 4A, 4B, 4C and 4D. Error bars are omitted for clarity. For articular cartilage mass removal and ablation efficiency are greatest at λ = 6.1 μm and the least at λ = 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 ($p < 0.05$). For fibrocartilage, 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 two wavelengths. Based on the data illustrated in Figure 4, 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 in that 6.1 μm ablation is more efficient than 2.9 μm ablation where water is the sole absorber [27,31]. 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].

The preliminary results of histologic evaluation on articular and fibro-cartilage for three different wavelengths are shown in Figure 5.

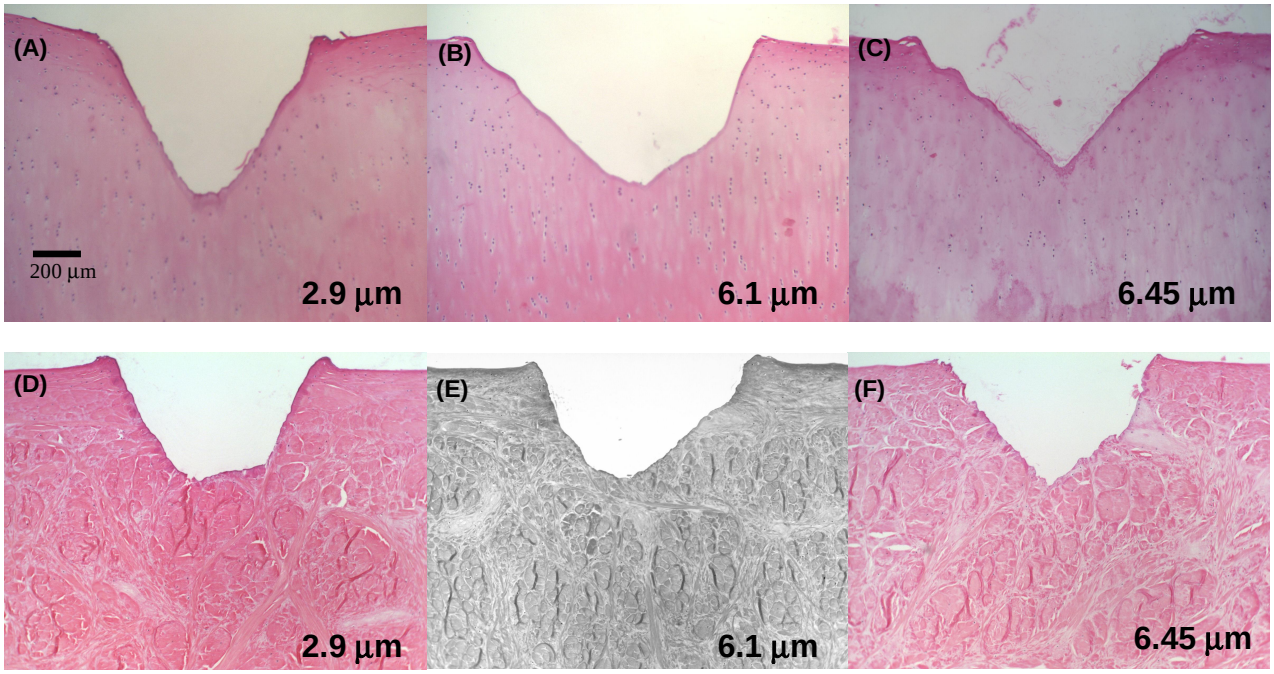

Figure 5. Histologic sections of articular (5A, 5B, and 5C) and fibro-cartilage (5D, 5E, and 5F) for three wavelengths.

Collateral thermal injury on the sides of the both cartilages at λ = 6.1 µm was less than those observed for other three wavelengths as seen in Figure 5. However, the collateral thermal injury of cartilage delivered by the selected FEL wavelengths was minimal in all cases (less than 20 µm). Further statistical evaluations of cut depth, width, area, and collateral thermal injury should be performed to investigate the relationship between the histology and the ablation efficiency presented here. Furthermore, correlation between the zone of collateral thermal injury and optical absorption characteristics of the principle components of cartilage should be investigated.

4. CONCLUSION

This study has shown that both articular and fibro-cartilage are most efficiently ablated when using the 6.1 µm wavelength. We observed the lowest ablation efficiency of articular cartilage at λ = 6.45 µm, but no statistical difference was observed for fibrocartilage ablation at λ = 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 fibrocartilage 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 fibrocartilage which contains more collagen than articular cartilage. However, at λ = 6.45 µm where protein is the dominant absorber, more energy is required to ablate articular cartilage than fibrocartilage. Further investigation is needed to compare the mass removal and ablation efficiency observations to thorough histologic evaluation of collateral thermal injury and ablation surface effects.

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