

Measurement of small-signal absorption coefficient and absorption cross section of collagen for 193-nm excimer laser light and the role of collagen in tissue ablation

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A 193-nm ArF excimer laser transmission was measured at subablative fluence through varying strength solutions of dissolved collagen, yielding an absorption cross section of $1.14 \times 10^{-17} \text{ cm}^2$ for the peptide bond, which accounts for 96% of the total collagen attenuation that is based on additional transmission measurements through solutions of isolated constituent amino acids. The measured absorption cross sections, in combination with typical corneal tissue composition, yield a predicted corneal tissue absorption coefficient of $16,000 \text{ cm}^{-1}$. In addition, dry collagen films were prepared and ablation-rate data were recorded as a function of laser fluence. Ablation rates were modeled by use of a Beer–Lambert blow-off model, incorporating a measured ablation threshold and an absorption coefficient that are based on the measured collagen absorption cross section and the film bond density. The measured ablation rates and those predicted by the model were in very good agreement. The experiments suggest that collagen-based absorption coefficients are consistent with predicted corneal tissue ablation rates and previously observed dynamic changes in tissue properties under ablative conditions. © 2004 Optical Society of America

OCIS codes: 170.0170, 170.1020, 170.4470.

1. Introduction

Clinical excimer laser refractive surgery systems are continually evolving to improve in accuracy and precision and hence to provide better refractive results. A more complete understanding of the ablation process may help to realize the goal of better outcomes, but the physics and mechanisms of corneal ablation and the role of the various tissue constituents are still not completely understood. At a more basic level, an understanding of the chemical composition of collagen, which is the primary constituent of corneal tissue, and its role in UV photochemistry is necessary.

A. Laser Light Absorption and Tissue Ablation

There have been a number of recent literature reviews that address the mechanisms and chemistry of

excimer laser tissue ablation.^{1–3} Although much has been learned to date about tissue ablation, notably at the 193-nm wavelength characteristic of the ArF excimer laser, a careful review of the literature reveals the lack of a comprehensive model that can be used to explain or predict tissue ablation rates with a high degree of accuracy. Many have suggested and some research has supported the idea that corneal tissue absorbs 193-nm ArF excimer laser light according to the Beer–Lambert law, meaning that the intensity of the light decays exponentially with depth into the tissue.^{1–5} The Beer–Lambert law is described by

$$I(x) = I_0 \exp(-\alpha x), \quad (1)$$

where I_0 is the laser intensity incident at the tissue surface, $I(x)$ is the intensity of the laser after it penetrates to tissue depth x , and α is the tissue absorption coefficient (usually reported in inverse centimeters) for the particular wavelength of laser light. Beer–Lambert behavior is certainly a valid approach for low-power irradiation, in which the incident energy density is well below the ablation threshold. However, under ablative conditions,

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Received 18 February 2004; revised manuscript received 11 May 2004; accepted 18 June 2004.

0003-6935/04/295443-09\$15.00/0

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nonlinear optical effects may alter the nature of the absorption coefficient. Notwithstanding such effects, the Beer–Lambert law can be used to provide a first-order estimate of ablation depth in what is often referred to as a “blow-off” model. In this model the depth of tissue ablation for a single laser pulse is equal to the depth at which the laser intensity has decayed to the threshold required for ablation. Defining I_{th} as the ablation threshold intensity, with incident intensity I_0 , the blow-off model predicts an ablation depth equal to

$$d_{\text{ablation}} = \frac{1}{\alpha} \ln\left(\frac{I_0}{I_{\text{th}}}\right). \quad (2)$$

The above analysis is coupled strongly to the corneal tissue absorption coefficient, which for 193-nm excimer laser light is perhaps one of the largest sources of controversy and the focus of much research. One of the first values reported for the tissue absorption coefficient, determined by measuring the transmission of excimer laser light through thin human and bovine corneal sections, was 2700 cm^{-1} at 193 nm.⁶ This value was reiterated and further supported by subsequent research,⁷ including a similar study with human and porcine cornea that yielded an average value of 2410 cm^{-1} .⁸ However, by the mid-1990s, researchers began to reexamine this issue. In 1993 Bor *et al.*⁹ reported a value of $20,370 \text{ cm}^{-1}$ for porcine corneal tissue that was based on fitting a logarithmic curve to a plot of ablation-rate versus laser-fluence (energy per unit area) measurements.⁹ By directly measuring corneal tissue reflectance and applying Fresnel theory to determine the complex refractive index of the tissue, Pettit and Ediger reported an absorption coefficient of approximately $40,000 \text{ cm}^{-1}$ for bovine cornea.¹⁰ Yablon *et al.* measured the absorption coefficient of bovine cornea by using a novel technique called interferometric photothermal spectroscopy and reported a value of $19,000 \text{ cm}^{-1}$.¹¹

The values reported by researchers in more-recent literature are an order of magnitude larger than the absorption coefficient first reported by Puliafito *et al.* in 1985.⁶ Pettit and Ediger contend that the absorption coefficient must be on the order of $20,000 \text{ cm}^{-1}$ based on theoretical considerations, namely by assuming that the peptide bond is the primary chromophore in corneal tissue for 193-nm light and by translating the bond density into an absorption coefficient, as well as on experimental evidence by measuring ablation-rate values as a function of laser fluence.¹⁰ However, consideration of a single absorption coefficient makes the implied assumption of a static and unchanging value during the ablation event, which may be an oversimplification.

Many researchers have suggested that the absorption coefficient is actually a dynamic quantity that may be enhanced under ablative conditions as

well as be influenced by matrix effects such as tissue hydration. It is generally accepted that there is a static “small-signal” absorption coefficient that remains constant for laser fluences below the ablation threshold. Some research has shown, however, that there are transient decreases in both corneal tissue reflectivity and transmission through the tissue under ablative conditions.^{12–16} Such data suggest that there is a transient effect that dynamically changes the absorption coefficient of corneal tissue during the ablation process, notably during the time course of the excimer laser pulse. Furthermore, research suggests that tissue hydration may play a role in transient changes in tissue absorptivity, possibly owing to the direct absorption of laser energy by water molecules and their subsequent vaporization.^{12,17,18} Alternative views are that ablation events at high laser fluence induce radical-species formation with high absorption cross sections, while also causing boiling of the water fraction of the cornea, resulting in changes in the overall absorptivity of the tissue.¹⁹ Electron paramagnetic resonance studies have corroborated the idea that reactive free-radical species are created during excimer laser ablation, suggesting that these radicals may play a significant role in laser–tissue interactions.²⁰

Despite the large body of research to date, no study has systematically investigated the absorption properties of individual corneal tissue constituents (namely collagen and water) by use of direct measurements. Furthermore, no clear and comprehensive description of the corneal tissue laser absorption and ablation processes has been developed. The current study is intended to further elucidate the role of corneal constituents, primarily collagen, in the ablation process.

B. Collagen Structure

To date, no published study has thoroughly described the structure of collagen (the primary constituent after water) in corneal tissue as it relates to photochemistry. Some presentations include discussions of basic collagen structure, but these treatments are generally cursory.¹⁹

For purposes of the present paper, the overall structural unit of collagen is referred to as the macromolecule. The collagen macromolecule is a right-handed, triple-helix structure, in which each helical strand is composed of a series of amino acids. The strands are linked together by bonds known as covalent cross-links, which provide structural integrity. The sequence of amino acids in a particular strand is a quasi-repeating pattern, typically represented as Gly-X-Y, in which glycine (Gly) appears as every third residue, X is generally considered proline, and Y is generally considered hydroxyproline. Other amino acids may appear in trace amounts in place of the proline and hydroxyproline, but collagen is most conveniently modeled as a repeating sequence of gly-

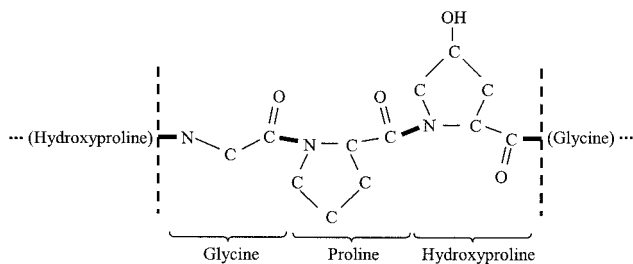


Fig. 1. Amino acid sequence constituting the primary structure of collagen ($C_{12}H_{17}N_3O_4$). Bold lines indicate peptide bonds (C-N) between adjoining amino acids.

cine, proline, and hydroxyproline in overall equal proportions. The chemical formulas for glycine, proline, and hydroxyproline are $C_2H_5NO_2$, $C_5H_9NO_2$, and $C_5H_9NO_3$, respectively. The three amino acids are linked to form the repeating sequence found in collagen, as depicted in Fig. 1.²¹⁻²⁷

The collective chemical formula for this repeating sequence of three amino acids is $C_{12}H_{17}N_3O_4$, which sums to 267 atomic mass units (amu). Note that the peptide bonds are formed through a substituted amide linkage, which also forms a water molecule (i.e., dehydration); hence the total atomic mass is different from the sum of the respective amino acids. The average weight of a typical collagen macromolecule is approximately 308,300 amu, which corresponds to 1155 groups of the repeating amino acid sequence. It is generally accepted that the primary chromophore for a 193-nm excimer laser light in corneal tissue is the peptide bond (C-N) between adjoining amino acids.^{28,29} Accordingly, one may calculate a total of 3465 chromophores for 193-nm radiation present in each macromolecule of collagen, based on three peptide bonds per repeating amino acid sequence (i.e., glycine-proline-hydroxyproline) and 1155 sequences per macromolecule. Quantification of the absorption characteristics of the purported peptide-bond chromophore is a significant goal of this paper.

2. Experimental Methods

A. Dissolved Collagen and Amino Acid Solutions

Transmission of 193-nm ArF excimer laser light at a subablative fluence of approximately 4 mJ/cm^2 was measured through four solutions, namely water, acetic acid, dissolved collagen in acetic acid, and dissolved amino acids in acetic acid. The transmission was measured by use of a fast-response (200-ps rise time) photodetector and a digital oscilloscope (2.5 gigasamples/s), through an UV grade cell (Suprasil 312) with an average path length of 0.167 cm. Laser line filters at 193 nm were used in front of the photodetectors for all experiments to eliminate spurious signals due to fluorescence, which was noted to be significant if the filters were removed. The transmission cell was constructed by pressing two 50-mm-diameter quartz flats together with a thin O-ring

between them. The O-ring sealed the desired solution between the two flats and provided a repeatable spacing (i.e., cell path length) between the two flats. The exact optical path length was determined by the difference between the measured thickness of the cell and the measured total thickness of the two glass flats alone (i.e., no O-ring). These measurements were made with a precision micrometer with an uncertainty of $\pm 12 \text{ }\mu\text{m}$.

For each solution transmission measurement, an average laser waveform was recorded first without the cell present, then through the cell containing the appropriate solution, then again without the cell present. Neutral-density filters were used whenever necessary to maintain signal linearity over a finite range. Transmission was calculated as the ratio of the integrated waveform (full peak area) recorded through the cell to the average of the two integrated waveforms recorded without the cell present, correcting for any differences in optical density that were due to neutral-density filters as necessary.

Pure deionized water was used as a control to verify repeatability as well as to assess the validity of the measurements based on the widely reported absorption coefficient of water at 193 nm. Collagen solutions were created by dissolving appropriate amounts of solid type III calfskin collagen (Sigma Chemical product C-3511) in a 0.5 N acetic acid solution. A stock solution was created by using 32 mg of collagen and 100 mL of acetic acid solution and stirring the resulting solution for 48 h.

After 48 h, transmission measurements were performed by use of the following experimental procedure. A laser-pulse waveform, the average of 200 individual laser pulses, was recorded without the cell present. The cell containing deionized water was then placed in the beam path, and a 200-pulse average waveform was recorded. The cell was removed, and a third 200-pulse average waveform was recorded. This process was repeated, but the middle waveform was recorded with the pure 0.5 N acetic acid solution in the cell. The process was repeated a third time, but with the middle waveform recorded with the desired solution strength of dissolved collagen in the cell. The collagen solution measurements were repeated in triplicate for each specified concentration; however, the acetic acid and deionized water measurements were recorded only once for each set of measurements. The process was repeated for serially diluted collagen solutions over the desired range, namely 0.24, 0.18, 0.12, and 0.06 mg/ml.

To assess the degree to which the peptide bond is the primary chromophore for 193-nm radiation, additional transmission measurements were made by means of equivalent concentrations of isolated amino acids (i.e., contained no peptide bonds). These amino acid solutions were created by dissolving appropriate amounts of solid powders of glycine (Sigma Chemical product G7403), L-proline (Fluka product 81709), and *cis*-4-hydroxy-L-proline (Fluka product

56248) in 0.5 *N* acetic acid solution. Specifically, a stock solution of 5 mg/ml was created with nominally equal amounts (100 mg each) of glycine, proline, and hydroxyproline (300 mg of total amino acids) dissolved into 60 ml of acetic acid solution. The resulting solution was then stirred for 48 h. The stock amino acid solution was then diluted to produce final concentrations equal to 1.25, 1.0, and 0.75 mg/ml. For each solution concentration, transmission measurements were recorded as described above, including the deionized water and acetic acid reference measurements.

B. Dry Collagen Films

As with the dissolved collagen solution studies described above, type III calfskin collagen was dissolved in 0.5 *N* acetic acid to create a solution with a nominal concentration of 1 mg/ml. The collagen solution was stirred for approximately 48 h to completely dissolve all of the collagen. Once dissolved, approximately 5 ml of the collagen solution was deposited on each 50-mm-diameter quartz flat and allowed to dry for 48 h. Once dry, each resulting collagen film was ablated by use of the same pattern of 4 rows with 8 ablation sites in each row, for a total of 32 sites. The first and third rows were ablated from left to right, starting at the low-pulse energy value (approximately 0.9 mJ/pulse) and increasing the energy incrementally at each site to the high-pulse energy value (approximately 3.8 mJ/pulse). The second and fourth rows were ablated from left to right, starting at the highest-pulse energy and ending at the lowest energy. The ablation pattern was alternated between rows in an attempt to compensate for any variations in film thickness.

At each ablation site, the collagen film was subjected to 25 laser pulses, which was sufficient to completely perforate the film for all pulse energies, enabling the last few pulses of the sequence to be used for normalization of the transmission values. For each ablation site, the pulse-to-pulse transmission was calculated based on the signal normalization of the transmitted integrated pulse area divided by the integrated pulse area averaged over the final five laser pulses. As mentioned above, the final five pulses were always found to correspond to essentially complete removal of the collagen film; hence they represent the reference transmission value (i.e., no film). This procedure yields a pulse-to-pulse transmission value between 0 and 1 for the first 20 laser pulses. For each laser-pulse energy investigated, the transmission values for each of the 12 ablation sites (1 crater per energy level on each of 4 rows per film over a total of 3 films) were averaged together as a function of laser-pulse number.

After the films were ablated as described above, the edges of individual craters were scanned with a white-light interferometer at five distinct locations on each of the three films. The interferometer produced a two-dimensional profile, showing the top surface of the unablated collagen film and the bottom surface of the ablated crater. It is noted that the

bottom surface was actually the surface of the quartz flat because the films were fully ablated (i.e., completely removed) at the crater bottom. From the resulting interferometer maps, the average thickness of the collagen films was determined by use of the 15 individual measurements (5 sites on each of 3 films).

3. Results and Discussion

A. Dissolved Collagen and Amino Acid Solutions

The average transmission measured through the cell containing pure deionized water, after correcting for Fresnel losses at the cell boundaries, was calculated as 0.98 ± 0.01 (0.8% relative standard deviation). Using this value and the known 0.167-cm path length, the Beer–Lambert law was used to calculate the absorption coefficient of the water, yielding a value of $0.15 \pm 0.05 \text{ cm}^{-1}$ [31.5% relative standard deviation (RSD)], which is in excellent agreement with the expected value for deionized water at 193 nm. The rather large relative error is reflective of the near-unity transmission value for this path length and the logarithmic nature of the Beer–Lambert law.

Given that absorption coefficients are additive, the absorption coefficients of the acetic acid and collagen solutions were calculated directly from the Beer–Lambert law:

$$\alpha_{\text{acid}} = \alpha_{\text{water}} - \frac{\ln(\tau_{\text{acid}}/\tau_{\text{water}})}{L}, \quad (3)$$

$$\alpha_{\text{collagen}} = \alpha_{\text{water}} - \alpha_{\text{acid}} - \frac{\ln(\tau_{\text{collagen solution}}/\tau_{\text{water}})}{L}. \quad (4)$$

When determining the absorption coefficient of water, we had to account for Fresnel losses in order to calculate an absolute value. However, Fresnel losses are considered to be identical for all the different solutions and were therefore neglected in calculations of the absorption coefficients of acetic acid and collagen owing to the *ratio* of transmission values in Eqs. (3) and (4). By use of the average absorption coefficient for water, and the measured transmission values of water and acetic acid, the absorption coefficient of acetic acid was determined to be $30.1 \pm 0.3 \text{ cm}^{-1}$ (1% RSD) for the 0.5 *N* solution. The near-unity value of the water transmission and the corresponding large RSD of the water absorption coefficient do not adversely affect the precision of the acetic acid absorption coefficient owing to the nearly 200-fold increase in acetic acid absorption as compared with water, hence the excellent precision.

The absorption coefficient of collagen was determined by use of the average values of τ_{water} , α_{water} , and α_{acid} , as measured over multiple experiments, to reduce the collagen-solution data. Because each collagen-solution measurement yields a corresponding absorption coefficient, it was necessary to normalize the data by the actual collagen-bond density. The following relation was used to calculate the equivalent peptide-bond number density *N* (bonds/

cm³) for each collagen solution, based on the collagen macromolecule parameters described above:

$$N = (Xg/cm^3) \frac{(3465 \text{ bonds/macromolecule})(6.022 \times 10^{23} \text{ macromolecules/mole})}{(308,300 \text{ g/mole})}, \quad (5)$$

where X is the given collagen-solution mass concentration. Equation (5) permits correlation of the measured absorption coefficient of the dissolved collagen solutions as a function of the peptide-bond number density (i.e., chromophore density). A plot of these results is shown in Fig. 2.

The absorption coefficient is the product of the chromophore number density and the chromophore absorption cross section; hence the slope of the plot in Fig. 2 yields the absorption cross section of the peptide bond. Based on the Fig. 2 data, the effective absorption cross section of the peptide bond in collagen is approximately $1.19 \times 10^{-17} \text{ cm}^2$. However, the peptide bond may not be the only absorber present in collagen, because the amino acids themselves may also absorb a portion of the incident laser energy. As outlined below, this effective cross section may be corrected to account for the contribution of the amino acids.

Previous studies dating to the 1950s have investigated the absorption of UV light by proteins, which include amino acids and the peptide bonds that join them, as well as by isolated amino acids that contain no peptide bonds.²⁹ However, these studies have generally concentrated on radiation in the near-UV

range of 240–290 nm, because 193-nm radiation was of limited interest when the studies were performed.

Hence an additional goal of the current study is to investigate the absorptive properties of *isolated* amino acids (i.e., no peptide bonding) for 193-nm radiation to quantify the role of the peptide bond as the primary chromophore. By use of procedures and equations similar to those used for the dissolved collagen solutions, the recorded transmission measurements of the 1:1:1 glycine, proline, and hydroxyproline amino acid solutions revealed absorption coefficients equal to approximately 4% of the total absorption coefficients recorded for the dissolved collagen solutions after adjustment for mass concentration. These measurements permit the direct calculation of the relative contribution of the peptide bonds and the amino acids themselves to the measured absorption coefficient of collagen. Based on these results, the actual absorption cross section of the collagen peptide bond is approximately $1.14 \times 10^{-17} \text{ cm}^2$, or 4% lower than the value reported above, and the average absorption cross section of an amino acid in collagen is approximately $4.74 \times 10^{-19} \text{ cm}^2$. The above results have effectively separated collagen into its constituent chromophores—the peptide bonds, which account for nearly all of the absorption, and the amino acids. The total effective absorption cross section for collagen is then the sum of these two, or $1.19 \times 10^{-17} \text{ cm}^2$ per amino acid unit (i.e., amino acid plus peptide bond).

The collagen-absorption cross sections measured in the current study may be used to estimate the absorption coefficient of corneal tissue. Assuming that corneal tissue is approximately 20% collagen and 80% water, and then using a tissue density equal to water (1 g/cm^3), a collagen molecular weight and peptide-bond density as described above, and the collagen effective cross section of $1.19 \times 10^{-17} \text{ cm}^2$ per amino acid unit, results in an equivalent corneal tissue absorption coefficient of $16,000 \text{ cm}^{-1}$ at 193 nm. The corresponding absorption cross section of a water molecule is $4.5 \times 10^{-23} \text{ cm}^2$ based on the measured absorption coefficient (0.15 cm^{-1}), making the contribution of water to the tissue absorption coefficient negligible. It is noted that this value ($16,000 \text{ cm}^{-1}$) is based solely on the contribution of collagen as quantified in isolated solutions. However, actual tissue will undoubtedly contain additional conformational changes and may contain other chromophores such as glycosaminoglycans, as suggested earlier.⁶ Nonetheless, as stated in the introduction, research has supported the argument that the absorption coefficient of corneal tissue is of the order of $20,000 \text{ cm}^{-1}$ or more. In fact, Pettit and Ediger¹⁰ used mo-

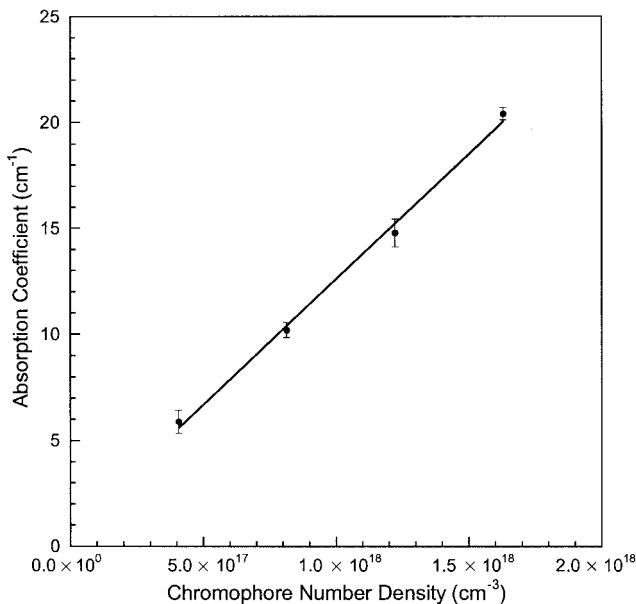


Fig. 2. Collagen absorption coefficient, with respect to 193-nm radiation, as a function of the number density of peptide bonds contained in solution. The error bars represent ± 1 standard deviation, and the solid line represents the least-squares fit line.

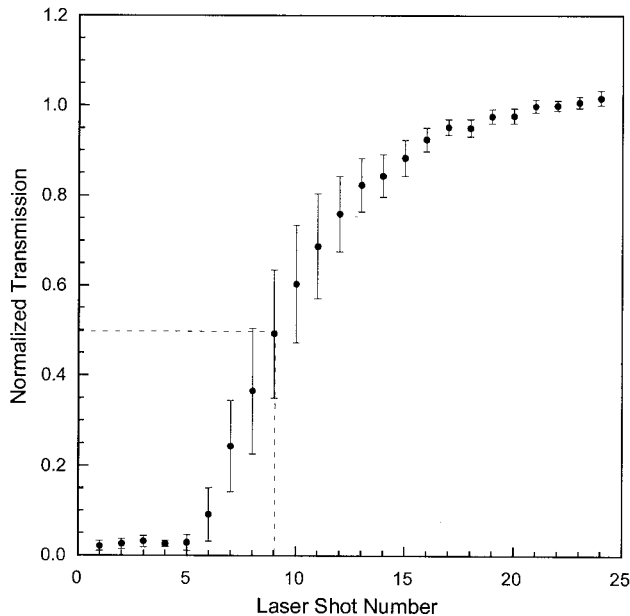


Fig. 3. Pulse-to-pulse progression of the transmission of 193-nm laser light through dry collagen films, normalized by the steady-state value reached after complete film perforation. The error bars represent 1 full standard deviation ($N = 12$). The dotted lines indicate the 50% transmission point and the corresponding number of laser pulses.

lar absorption coefficient data to estimate an equivalent corneal tissue absorption coefficient of $20,000 \text{ cm}^{-1}$, which is in very good agreement with the current value.

Notwithstanding this agreement, a key factor that must be considered in the context of corneal absorption coefficients that are relevant to excimer laser ablation is the nature of a static value as applied over a significant range of laser energies, including under ablative conditions. The value calculated in this study ($16,000 \text{ cm}^{-1}$) is truly a small-signal (i.e., subablative-condition) corneal absorption coefficient, which is likely to be enhanced as a result of laser-tissue interactions realized with significantly higher-pulse energy, notably within the ablation regime. Prior research has documented that there is in fact a dynamic enhancement in tissue absorption and reflection properties during 193-nm laser ablation, as discussed above. However, with previously reported corneal tissue absorption coefficients as low as 2400 cm^{-1} , it was difficult to reconcile the order of magnitude increases in absorption that are necessary for explaining ablation-rate data. Based on the current measurements of collagen absorption properties, the predicted corneal absorption coefficient of $16,000 \text{ cm}^{-1}$ necessitates only a modest dynamic enhancement (not an order of magnitude) to agree with the reported range of ablation-rate data. A comprehensive understanding of the laser-tissue interaction process under ablative conditions requires additional study; hence elucidating the role of collagen in the context of the measured absorption cross sections is a key step toward achieving this goal.

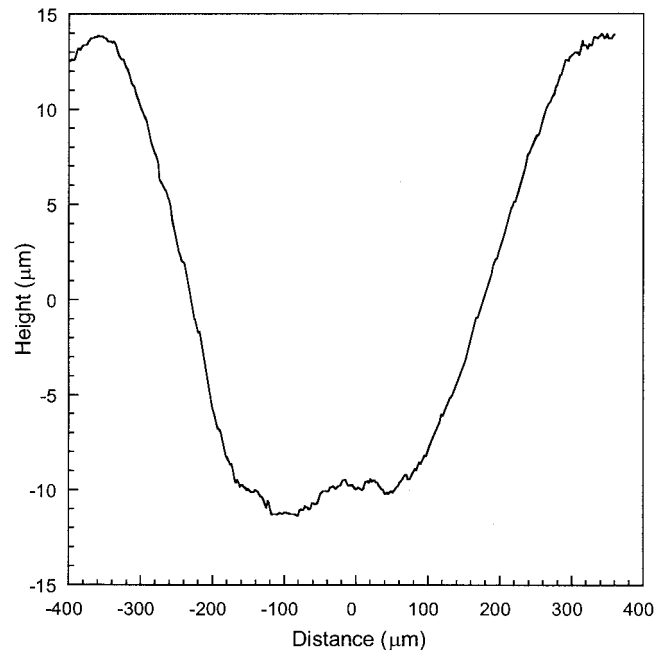


Fig. 4. Ablation crater showing ablation profile of the ArF excimer laser beam. The crater was recorded in bovine corneal tissue.

B. Dry Collagen Film Ablation

For each ablation site, following the first few laser pulses, the pulse-to-pulse normalized transmission was observed to increase monotonically to the asymptotic value of unity. The pulse-to-pulse progression of the normalized transmission is presented in Fig. 3 for a laser-pulse energy of approximately 0.9 mJ/pulse , as averaged over 12 different ablation sites. The average film thickness, as measured by white-light interferometry, was $3.2 \pm 0.5 \text{ μm}$ (15% RSD). The full film thickness was sufficient to initially render the film opaque to the incident laser pulse; hence the first few laser pulses resulted in zero transmission. Subsequent laser pulses steadily ablated through the full thickness of the collagen film.

As demonstrated by the Fig. 3 data, the laser energy was sufficient to perforate through and fully remove the films after a number of pulses, ultimately reaching a steady-state transmission value. By normalizing the pulse-to-pulse transmission values by the final five pulses for each film site, the slight variations in the pulse-to-pulse progressions between different ablation sites that are due to variations in film thickness are minimized. The monotonic rise between the nominally zero and unity transmission values are interpreted in terms of the ablation process and the laser beam profile. The laser beam profile produces a bullet-shaped ablation crater, as characterized in an earlier study³⁰; hence the ablation rate varies somewhat across the full laser beam diameter. For reference, an ablation profile recorded in bovine corneal tissue is presented in Fig. 4, as measured with a previously reported technique.³⁰ Therefore the initial rise in the pulse-to-pulse transmission following the first few pulses of nominally zero trans-

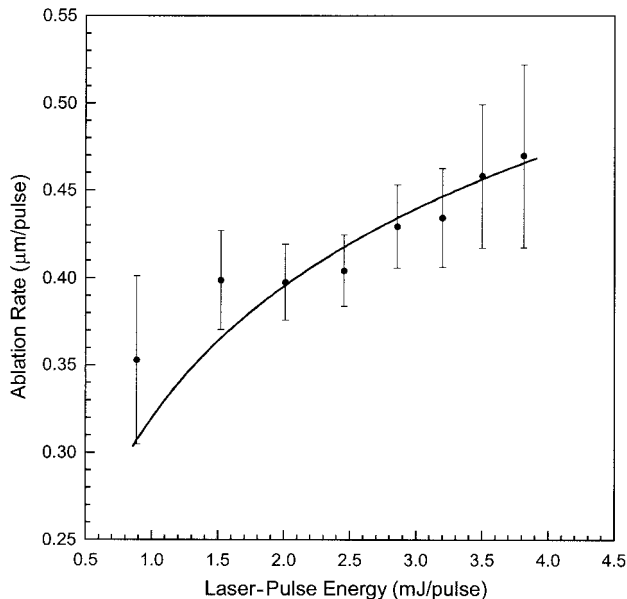


Fig. 5. Ablation rate through dry collagen films as a function of laser-pulse energy. The circles represent the experimental data, with the error bars indicating ± 1 standard deviation. The solid line represents predicted ablation rates based on a Beer-Lambert blow-off model that incorporates the experimentally measured absorption cross section of collagen.

mission is interpreted as the point at which the collagen film is sufficiently thinned such that a fraction of the incident laser pulse is transmitted through the film. The next few pulses are considered to fully remove the film first at the very center of the ablation site, with subsequent pulses gradually widening the zone of complete film removal until the ablation crater approaches a diameter equal to the full beam size. As described above, pulse-to-pulse transmission data were recorded over 12 different sites by use of 3 different films for each energy level. The collagen film thickness was found to be quite consistent based on the repeatability of the ablation experiments and on the white-light interferometry data. In one case, the number of pulses for perforation was nearly twice the value measured for all of the other experiments at the same pulse energy. This single data point was considered the result of an uncharacteristically thick local section of the film and was omitted from further consideration.

The pulse-to-pulse progression of the transmission data was then used to calculate an equivalent ablation rate for each recorded laser-pulse energy. As shown in Fig. 3, the dotted lines indicate the point where the normalized transmission reached 50% of its steady-state value. The point of 50% transmission and the corresponding number of laser pulses needed to reach this value were used to define the characteristic ablation rate. Specifically, for each laser-pulse energy, the ablation rate was calculated as the average film thickness ($3.2 \mu\text{m}$) divided by the number of laser pulses required to produce a normalized transmission of 50%. The measured ablation

rate as a function of pulse energy is presented in Fig. 5 for the collagen films. The error bars reflect the average error in the number of pulses needed to achieve a 50% transmission value, as calculated with the error bars in the normalized transmission profiles (see Fig. 3). Over the nominal range of pulse energies from 1 to 4 mJ/pulse, the collagen film ablation rates were found to vary from approximately $0.35 \mu\text{m}/\text{pulse}$ to greater than $0.45 \mu\text{m}/\text{pulse}$. To avoid ambiguity associated with a laser beam profile, the ablation-rate data are reported as a function of total pulse energy rather than of laser fluence. The average fluence is readily calculated from the measured full laser spot size of approximately 0.8 mm^2 .

C. Ablation-Rate Model for Collagen

To explore the role of collagen absorption characteristics, namely the peptide-bond cross section, in excimer laser ablation, the ablation rates of the collagen films were modeled with the Beer-Lambert blow-off model. The blow-off model, as given by Eq. (2), requires an effective absorption coefficient of a collagen film and an ablation threshold energy to predict the ablation depth as a function of pulse energy. The ablation threshold was experimentally determined for the same collagen films by incrementally decreasing the laser-pulse energy to determine the value at which point the collagen film remained intact, regardless of the number of laser pulses. Lack of ablation was verified by the absence of transmission through the film for hundreds of laser pulses. Based on repetitive trials, the average ablation threshold was determined to be $0.053 \text{ mJ}/\text{pulse}$. This experimental value was then used for the Beer-Lambert blow-off model. When the measured full beam diameter is used this value corresponds to an average threshold fluence of $\sim 10 \text{ mJ}/\text{cm}^2$, which is in the range of values expected for tissue and collagen. The peak fluence is expected to be 2 to 3 times greater based on the actual beam profile.

Because absorption coefficients are additive, the absorption coefficient of a collagen film was calculated as the sum of the absorption contribution of the peptide bonds and the contribution of the amino acids in the collagen, with the former equal to the product of the peptide-bond absorption cross section and the peptide-bond number density, and the latter equal to the product of the amino acid absorption cross section and the amino acid number density. For the collagen structure, the peptide-bond number density is assumed to be equal to the amino acid number density, although the total number of peptide bonds is three less than the total number of amino acids owing to the triple-stranded structure. The peptide-bond number density in a collagen film is equal to the absolute number of peptide bonds divided by the total volume of the film. The absolute number of peptide bonds was calculated as the product of the peptide-bond number density in the solution used to prepare the film [see Eq. (5)] and the volume deposited to form the film (5 ml). The surface area of a dry collagen film was not measured directly, owing to the film's

transparent nature, approximate 3- μm thickness, and irregularly contoured edges. However, careful examination in reflected light revealed that the films were certainly smaller than the entire area of the quartz flats (50-mm diameter), but were considered larger than the area defined by the inner half-diameter (i.e., 25-mm diameter) of a quartz flat. Working within these two limiting areas, we estimated the total surface area of a dry collagen film to be two-thirds of the total available area of the quartz flat; hence the surface area was equal to 13.5 cm². By use of the measured film thickness, the average total volume of a collagen film was therefore estimated to equal 0.004 cm³.

Each collagen film was prepared with 5 ml of a collagen solution at a concentration of 1 mg/ml, which when combined with Eq. (5) and the total volume of a dry film, yields a peptide-bond density of approximately 7.8×10^{21} cm⁻³. Using the respective absorption cross sections of the peptide bonds and the amino acids yields a collagen film absorption coefficient of approximately 91,900 cm⁻¹.

Finally, using the measured ablation threshold of 0.053 mJ/pulse and the calculated absorption coefficient of 91,900 cm⁻¹, the Beer-Lambert blow-off model [see Eq. (2)] was applied over the range of laser-pulse energies that correspond to the dry film ablation study in order to predict the ablation rates. The results are shown as the continuous curve in Fig. 5, which permits a direct comparison with the experimentally determined ablation rates. It is noted that because Eq. (2) utilizes the ratio of incident and threshold intensities, identical results are obtained whether absolute pulse energy or laser-pulse fluence is used. The agreement between the experimental data and the results of the Beer-Lambert model is considered to be very good, given the independent measurement of the ablation threshold, the direct calculation of the absorption coefficient from independently measured absorption cross sections, and the somewhat arbitrary definition of the experimental ablation rate as based on the 50% transmission point of the pulse-to-pulse transmission profiles. A point-to-point comparison of the predicted ablation rates and the experimental data over all data points yields an average difference of 3.8%.

4. Summary and Conclusions

A key finding of the current study is the direct measurement of the 193-nm absorption cross section of collagen, equal to 1.19×10^{-17} cm² per amino acid unit. Furthermore, it has been demonstrated that the peptide bond between adjoining amino acids is responsible for approximately 96% of the absorption, which yields an absorption cross section of 1.14×10^{-17} cm² per peptide bond. The remaining 4% of collagen absorption is due to the amino acids themselves, corresponding to an average amino acid absorption cross section of 4.74×10^{-19} cm². This study is believed to be the first to directly quantify the peptide bond as the primary collagen chromophore for 193-nm laser radiation. Previous research sug-

gests that, in general, peptide-bond absorption may be dependent on the actual conformation of the overall protein.²⁹ The study of such effects is beyond the focus of the current study; nonetheless, the conformation of the collagen used in the current experiments is considered to be representative of the collagen present in corneal tissue.

For dry collagen films, a Beer-Lambert blow-off model was formulated by use of the measured peptide bond and the amino acid absorption cross sections, the estimated collagen film bond density, and the measured ablation threshold. The corresponding absorption coefficient of the dry collagen films was calculated as approximately 91,900 cm⁻¹, which yielded a very good agreement between the Beer-Lambert model and the experimental ablation-rate data. Such agreement is considered strong evidence to support the measured absorption cross sections of collagen and, more important, the role of the peptide bond in collagen ablation. It is noted that the agreement between the model and the experiments is based on a static absorption coefficient, which is believed to be appropriate for the dry collagen films. This finding is consistent with previous work with corneal tissue that shows that tissue hydration plays a role in the dynamic changes in tissue optical properties under ablative conditions.¹³

Measurement of the actual constituent absorption cross sections permits calculation of the equivalent absorption coefficient of corneal tissue that is based on a tissue composition of 20% collagen and 80% water. The result is an absorption coefficient equal to 16,000 cm⁻¹ for corneal tissue at 193 nm. This value is much larger than early measurements of 2400–2700 cm⁻¹, but on the same order of magnitude as more-recent estimates in the range 20,000–40,000 cm⁻¹.^{6–11} Although measured at subablative fluences, these higher values can be used in a Beer-Lambert blow-off model to predict ablation rates that are consistent with experimentally measured rates for a range of data,¹⁰ whereas the lower value (2700 cm⁻¹) grossly overestimates the rate of corneal tissue ablation. However, both cases should be considered in the context of ablation-induced laser-tissue interactions; namely, dynamic changes in optical properties that are manifested as transient changes in tissue absorptivity and reflectivity, as discussed above. The reconciliation of the observed ablation-rate data, the observed transient changes in tissue properties, and the range of corneal tissue absorption coefficients as reported in the literature may be explained by (i) an extremely high corneal tissue absorption coefficient ($\sim 30,000$ cm⁻¹) that remains essentially static such that tissue ablation is well described by a Beer-Lambert blow-off model or (ii) tissue ablation effects that produce an order-of-magnitude enhancement in the absorption coefficient from the small-signal value (i.e., $\sim 3,000$ to 30,000 cm⁻¹) as a result of laser-tissue interactions. Neither explanation is completely satisfactory, given that excimer laser tissue ablation almost certainly causes a dynamic enhancement in tissue absorption

in view of the large body of experimental evidence; however, an order-of-magnitude transient enhancement is difficult to explain. Clearly the current state of corneal tissue ablation modeling is too primitive to offer a definitive assessment. However, the current study supports a view that is between these two limiting cases; namely, a small-signal absorption coefficient of corneal tissue that is equal to approximately $16,000 \text{ cm}^{-1}$ for 193-nm radiation, as based on the measured peptide bond and amino acid absorption cross sections from isolated solutions. This value is consistent with the reported range of ablation-rate data under the assumption of a modest dynamic increase of 25% to 75%. Such a degree of dynamic change is consistent with the need for some transient perturbation in tissue properties in order to be consistent with experimental observations and is also more acceptable than an order-of-magnitude change under modest ablative conditions. Overall, the current study further elucidates the role of collagen, notably the peptide bonds, in excimer laser tissue ablation, although the exact nature of these laser-tissue interactions remains in need of additional investigation.

This research was supported in part by a grant from Alcon Research, Ltd. The authors thank Greg Sawyer for assistance with the white-light interferometry measurements.

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