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ABSTRACT

Approximately 5 million people worldwide are blind due to complications from glaucoma. Current surgical techniques often fail due to infection and scarring. Both failure routes are associated with damaging the surface tissues. Femtosecond lasers allow a method to create a highly precise incision beneath the surface of the tissue without damaging any of the overlying layers. However, subsurface surgery can only be performed where the beam can be focused tightly enough to cause optical breakdown. Under normal conditions, subsurface surgery is not possible since sclera is highly scattering. Using two independent methods, we show completely subsurface surgery in human sclera using a femtosecond laser. The first method is to make the sclera transparent by injecting a dehydrating agent. The second method is to choose a wavelength that is highly focusable in the sclera. Both methods may be applied in other tissues, such as skin. We show highly precise incisions in in vitro tissues. Subsurface femtosecond photodisruption may be a useful for in vivo surgical technique to perform a completely subsurface surgery.

Key words: sclera, glaucoma, photodisruption, femtosecond, laser, skin, inducing transparency

1. INTRODUCTION

A 1995 World Health Organization study reported that over five million people worldwide were blind from complications due to glaucoma. Some 2.4 million people develop glaucoma a year and another 100 million develop a significant elevated intraocular pressure (IOP) putting them at risk for glaucoma. Glaucoma occurs in about 1.5-2.0% of the general population and in 5% of those of African descent. Treatment and loss in productivity are estimated to cost the United States over $2.5 billion a year. Glaucoma has no cure, and treatments generally become ineffective over time. Femtosecond lasers offer a fast and novel surgical method for treating glaucoma that is not possible by other means. This paper describes the methods and parameters for subsurface femtosecond laser glaucoma surgery and skin surgery.

Glaucoma describes a class of diseases that involve optic neuropathy (optic nerve damage) and visual field loss. Typically, glaucomas are accompanied by an increase in intraocular pressure (IOP). Most treatments for glaucoma have focused on reducing the intraocular pressure. In the most common surgical procedure, a drainage passageway is cut through the sclera to relieve pressure as shown in Figure 1. This surgery generally fails over time due to scarring of the overlying tissues, which may be instigated by the mechanical manipulation used to make the incision.
Femtosecond lasers provide a way to cut beneath these tissues without damaging them. At the focus of a femtosecond laser pulse, the intensities are so great that matter is torn apart and transformed into a plasma in a process known as photodisruption. Since photodisruption only occurs at the focus, a completely subsurface surgery is possible. In contrast, conventional lasers remove tissue only at the surface. Femtosecond photodisruption produces subsurface micron size ablations with virtually no collateral damage.

Focusing beneath the surface in a transparent material, such as the cornea, is straightforward. However, glaucoma surgery must be performed in the highly scattering sclera. Under normal circumstances, scattering prevents the tight focus needed for photodisruption. In this paper, we have overcome scattering in using two methods. The first method is to make the sclera transparent by injecting a dehydrating agent. The second method is to choose the wavelength so that the beam is not scattered or absorbed before focus. Using either or both of these methods will allow for a glaucoma surgery to be performed completely beneath the tissue surface. Similar techniques may also be used in skin.

2. EXPERIMENTAL SETUP

2.1 Tissue

Human globes, not suitable for transplantation, obtained 1-3 weeks after harvesting. Sclera samples, about 5x10mm, were cut from just behind the limbus. The samples were cleaned: surface conjunctiva and brown matter were removed. The samples were then soaked in physiological saline solution to obtain full hydration. Hypaque 76\textsuperscript{6} was used to induce transparency in the examples shown in Section 3. Fully hydrated samples were used in Section 4.

Yearling pigskin was obtained two days after harvest. The skin was shaved and about 2 mm thick pieces were cut from the epidermis downward. To induce transparency, glycerin was used.\textsuperscript{7}

2.2 Apparatus

The tissue was mounted between two microscope slides. Pressure was sufficient to flatten the tissue but not enough to see a change in transparency.\textsuperscript{8} The mount was then attached to a three-axis computer controlled translation stage to scan the desired patterns as shown in Figure 2. In all cases, the beam was focused with a 0.5 NA aspherical lens (ThorLabs C240TM). A home built -150 fs, 775 nm, 1.3 mJ, 1 kHz Ti:Sapphire laser system was used to cut tissue with induced transparency. This laser was also used to pump an Optical Parametric Amplifier (TOPAS, Light Conversion/Quantronix) to provide the 1700 nm \textasciitilde130 fs pulses. Spot sizes, measured using a 40X microscope objective, were approximately 1.5 microns full-width half-maximum for 775 nm light and about 3 microns full-width half-maximum for 1700 nm light.
Figure 2. Laboratory apparatus used to cut the tissue. The tissue was mounted between two microscope slides (1mm thick) and scanned in the focus of a femtosecond beam used a computer controlled three-axis translation stage.

3. TRANSPARENCY INDUCED PHOTODISRUPTION

3.1 Inducing Transparency

It has been known for many years that dehydration makes the sclera transparent. This can be achieved by injecting a dehydrating material into the sclera, such as Hypaque, as shown in Figure 3. The effect is reversible and has been applied in vivo. Pressure also achieves a similar dehydrating effect.

Figure 3. Inducing transparency in sclera. (a) Hypaque was used to induce transparency in human sclera. (b) The tissue returns back to its initial state after rehydration.

3.2 Human Sclera

The tissue was soaked for 20 minutes in Hypaque 76, which is the maximum time for a reversible effect in sclera, to induce transparency. A 775 nm femtosecond laser was used to cut the two representative examples shown in Figure 4 and Figure 5. Extremely precise, sub-10 micron incisions, were made with no apparent collateral damage throughout the bulk of the sclera.
Figure 4. Femtosecond laser created transscleral channel in dehydrated sclera. (a) A channel with a minimum width was demonstrated. The channel width is estimated at 10 microns or less from this cross-section. A plane was scanned in order to find the channel upon dissection. (Laser parameters: 4.2 μJ, 150 fs, 775 nm, 1 kHz, 2 μm FWHM spot, 1.5 μm spot separation.) The minimum width channel was made through human sclera. (b) Potential application of creating a drainage passageway. (c) Scan pattern.

Figure 5. Channel for presbyopia implants. (a) The pocket was easily opened with forceps and a pin was placed inside. (b) Close up of the incised interface. (Laser parameters: 6 μJ, 150 fs, 775 nm, 1 kHz, 1.5 μm FWHM spot, 5 μm spot separation.) (c) A subsceral pocket is created 3 mm posterior to the limbus. (d) Raster pattern for the pocket.
3.3 Pig Skin

Transparency can also be induced in skin. We induced transparency in fresh pig skin using glycerin. Hypaque 76 was not as effective in inducing transparency. It took Hypaque an hour to see any clearing, whereas glycerin could be seen working in minutes. An incision was created in Figure 6. This incision appears to have a channel width less than 30 microns. Air bubbles were seen at depths greater than 2 mm. However, the surgical effect indicated from the air bubbles did not appear on SEM. In addition, these incisions could be opened by pulling apart with a forceps. This may suggest some tissue weakening and not a continuous incision was achieved or scattering may have only allowed focusing at sporadic locations.

![Figure 6. Channel cut in dehydrated pigskin. A 775 nm laser was used to cut an incision into the tissue. Even though air bubbles were apparent >2mm into the tissue, only about 0.5 mm of penetration was seen. In untreated skin, the beam is not seen to penetrate more than 50 microns. (Laser parameters: 100 µJ, 150 fs, 775 nm, 1 kHz, 1.5 µm FWHM spot, 3 µm spot separation.)](image)

4 LONG WAVELENGTH PHOTODISRUPTION

Transmission of human cornea and sclera is shown in Figure 7. Maximum forward or axial transmission — that emerging along a narrow cone of the incident light — occurs at 1700 nm according to these spectrophotometer measurements whereas total transmission — that emerging from the tissue at all angles — peaks between 1100-1300 nm. The discrepancy in the peaks implies that sclera is highly scattering. The best hope of achieving the subsurface focusing needed photodisruption will probably occur at the longest possible wavelength, since scattering decreases with increasing wavelength. The absorption peaks of water, seen at 1450 nm, 1900 nm, and >2400 nm, also restrict the choice of wavelength. For this experiment, 1700 nm light was selected since it has the maximum axial transmission through sclera, and less absorption than 2200 nm, which may lead to thermal damage.
Figure 7. Total and axial transmission through human cornea and sclera. ‘Total’ transmission curves capture all light emergent from the tissue with an integrating sphere, whereas the ‘Axial’ transmission curves only measure the wide beam undeviated light. Cornea scatters light since the total and axial transmission are not identical. In the sclera, axial transmission increases with increasing wavelength. For both tissues, scattering becomes more forward directed with increasing wavelength. Water absorption peaks are evident at 300 nm, 1450 nm, 1900 nm, and 2500 nm. Curves were adapted. 

4.1 Spot-size Measurements

To confirm the implications of Figure 7, we experimentally measured the spot using a 40X objective attached to a vidicon camera (Electrophysics 7290A-06). The beam was focused through fully hydrated (white) sclera using a 0.5 NA aspherical lens. The lens was translated along the axis of the beam until the smallest spot was obtained. At 775 nm, the spot is heavily scattered. As the wavelength increases, the spot becomes smaller. The smallest spots appear at 1700 nm and 2200 nm, and possibly at 1300 nm. Significant scattering appeared even at these wavelengths, but the focus may be satisfactory enough to achieve a transscleral procedure.

\[ \lambda = 775\text{nm} \quad \lambda = 1100\text{nm} \quad \lambda = 1300\text{nm} \quad \lambda = 1700\text{nm} \quad \lambda = 2200\text{nm} \]

Figure 8. Minimum spot size obtainable on back surface of sclera. Low power laser beams were focused through 0.5 mm thick human sclera with a 0.5 NA aspheric lens. The inset in the upper left corner shows the smallest spot obtainable through a 0.5 mm thick slab of water. The square in the bottom right corner is 10 microns. The beam is not focusable at 775 nm. As the wavelength increases, the spot size decreases.
4.2 Long-wavelength Subsurface Photodisruption in Human Sclera

Figure 9 shows the depth of penetration of 775 nm and 1700 nm in sclera. A stair step pattern was cut in order to look for damage above the desired ablation site. The 775 nm light only penetrates to 250 microns at most, whereas the 1700 nm light penetrated through the sclera and into the glass substrate behind the tissue. In addition, no damage occurred above the ablation sites in both cases. Using the 775 nm light, higher energies resulted in damage on the surface and in the bulk. At 80 μJ and 775 nm (not shown), the ablation severely damaged the tissue, but it did not penetrate to the back surface.

Several examples at 1700 nm were cut. Figure 10 shows a back surface ablation of fully hydrated sclera using 1700 nm. No damage appeared above the damage site. Figure 11 shows a 10 micron wide transcleral channel. Figure 12 shows a completely subsurface vessel that may be useful in fluid collection. Figure 13 shows a plane cut parallel to the surface using 1700 nm light. The incision was opened, and a pin was placed inside to prove that the incision was continuous.

Figure 9. Stair pattern cut in human sclera using (a) 775 nm and (b) 1700 nm. The scanned pattern was the same in both samples. Tissue thickness was 0.5 mm before SEM preparation, which results in significant tissue shrinkage. Each step is 50 microns deeper into the tissue. 775 nm light only penetrated up to 250 microns into the tissue, whereas 1700 nm light penetrated through the entire thickness. (Laser parameters: 10 μJ, 130 fs, 1 kHz, 5 μm spot separation, 50 x 50 μm rectangles were scanned.)
Figure 10. Back surface ablation in fully hydrated human sclera using 1700 nm laser. Back surface photodisruption was achieved without damaging the tissue above. (Laser parameters: 8 μJ, 130 fs, 1700 nm, 1 kHz, 3 μm FWHM spot, 3 μm spot separation.)

Figure 11. Transcleral channel cut from back surface to front. Channel is about 10 microns wide. Distortion occurs due to SEM processing. (Laser parameters: ~5 μJ, 130 fs, 1700 nm, 1 kHz, 3 μm FWHM spot, 2 μm spot separation.)
Figure 12. Subsurface vessel (artificial Schlemm’s Canal) created in sclera using 1700 nm light. (a) SEM, (b) potential surgery, (c) scan pattern. (Laser parameters: ~8μJ, 130 fs, 1770 nm, 1 kHz, 3 μm FWHM spot, 2 μm spot separation.)

Figure 13. Channel for presbyopia implant created in fully hydrated (white) human sclera using 1700 nm laser. See Figure 5 for a comparison in dehydrated (clear) sclera. (Laser parameters: 10 μJ, 130 fs, 1770 nm, 1 kHz, 3 μm FWHM spot, 2 μm spot separation.)

5 CONCLUSIONS

This paper describes a method for highly precise subsurface photodisruption in scattering tissues, namely the sclera. However, the methods described may be applied to other tissues, such as the skin. The essential feature of this work is how to reduce scattering in translucent tissues to achieve a tight enough focus for photodisruption with micron precision. Either the optical properties of the tissue can be changed by dehydration through pressure or the use of a dehydrating agent, or the wavelength can be selected to decrease scattering and maximize transmission. Subsurface photodisruption allows for a completely subsurface surgery, that is, no incision at the surface.
Subsurface femtosecond glaucoma surgery has the following advantages over current methods. (1) The beam can be focused beneath the easily scarred layers, thus preventing closure of the channel. (2) Any pattern with less than 10 microns precision can be cut in the bulk of the sclera by scanning the focus. (3) The procedure is completely computer controlled, thus eliminating the outcome of surgery on the surgeon’s skill. (4) The incision has very little collateral damage. (5) Total treatment time may be less than five minutes depending on the pattern to incise. (6) Since the surface is never broken, there is minimal risk of infection. Similar advantages are expected in skin and in other biological tissues.

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