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Optical Clearing of Vaginal Tissues in Cadavers

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Abstract

A nonsurgical laser procedure is being developed for treatment of female stress urinary incontinence (SUI). Previous studies in porcine vaginal tissues, ex vivo, as well as computer simulations, showed the feasibility of using near-infrared laser energy delivered through a transvaginal contact cooling probe to thermally remodel endopelvic fascia, while preserving the vaginal wall from thermal damage. This study explores optical properties of vaginal tissue in cadavers as an intermediate step towards future pre-clinical and clinical studies. Optical clearing of tissue using glycerol resulted in a 15–17% increase in optical transmission after 11 min at room temperature (and a calculated 32.5% increase at body temperature). Subsurface thermal lesions were created using power of 4.6 – 6.4 W, 5.2-mm spot, and 30 s irradiation time, resulting in partial preservation of vaginal wall to 0.8 – 1.1 mm depth.

Keywords

incontinence; laser; optical clearing agent; optical coherence tomography; thermal remodeling

1. INTRODUCTION

1.1 Female Stress Urinary Incontinence (SUI) and Conventional Treatments

Over 6.5 million women in U.S. suffer from stress urinary incontinence (SUI), but only ~200,000 women choose surgery [1,2]. The need for general anesthesia, long recovery time, incisions, potential treatment failures with future pregnancy, and procedural morbidity are some reasons for hesitation to have surgery. There is a role for a nonsurgical method to improve quality of life if it can be rapidly performed with minimal morbidity and short recovery.

1.2 Radiofrequency (RF) Thermal Treatment of Female Stress Urinary Incontinence

Radiofrequency (RF) energy has been used for transurethral thermal shrinkage and remodeling of submucosal collagen in the bladder neck and proximal urethra with success

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rates up to 80% [3,4]. However, RF therapy requires multiple needles inserted into the endopelvic fascia and is more invasive than desired by SUI patients.

1.3 Laser Treatment of Female Stress Urinary Incontinence

Our previous lab studies using a near-infrared (IR) laser and contact cooling probe demonstrated subsurface thermal denaturation of tissues with tissue surface preserved to 1–2 mm depth [5–8]. For SUI, the goal is to thermally remodel endopelvic fascia without damage to adjacent tissue (e.g. vaginal wall / urethra). The vaginal wall, endopelvic fascia, and urethral wall thicknesses are ~ 2.7, 4.3, and 2.4 mm [9,10]. Computer simulations of light transport, heat transfer, and tissue damage showed that a transvaginal approach is more feasible than a transurethral approach [11]. Further simulations were performed using glycerol as optical clearing agent (OCA) to provide deeper penetration of near-IR light into endopelvic fascia and greater preservation of vaginal wall [12].

1.4 Optical Clearing Agents

Optical clearing agents, including glycerol, are used for reducing light scattering and increasing optical penetration depth (OPD) in tissue through refractive index matching and dehydration [13,14]. The OCA induces water flux from interstitial space to tissue surface, and increased osmolarity of interstitial fluid draws water from the cell, leading to dehydration. The reduced scattering achieved with an OCA may enhance optical transmission, which in turn translates into improved OPD. The objective of this study is to explore optical properties of human vaginal tissue in a cadaver model as an intermediate step towards future pre-clinical and clinical studies, *in vivo*.

2. METHODS

2.1 Ultrasound Imaging Studies

Three cadaver studies were conducted to better understand the anatomical position of the treatment area and to test ergonomics of transvaginal laser probe. In the first cadaver study, an experienced gynecologist at Carolinas Medical Center performed ultrasound (US) scans to locate the treatment zone. A high-frequency, 13 MHz, endovaginal US system and probe (Flex Focus 400, anorectal 3D 2052) (Figure 1AB) measured thickness of vaginal wall, endopelvic fascia, and urethral wall. The main goal was to determine the ideal treatment zone location for transvaginal delivery via US. An US probe was deployed for the first cadaver to scan transvaginally with US transmission gel used to insure contact between US probe and tissue. The second cadaver study was used to examine depth of laser treatment region. The optimal position was at 12 o'clock when cadaver was in supine position and ~ 3 cm deep from the vaginal opening.

2.2 Reflection Spectroscopy

A spectrometer (USB 4000-VIS-NIR, Ocean Optics, Dunedin, FL) (Figure 1C) quantified change in the reflection spectrum of porcine vaginal wall as a function of OCA application time. A fiber optic Y cable (RP21, Thorlabs, Newton, NJ) was used to connect light source (MI-150, Edmund Optics, Barrington NJ), and spectrometer. The fiber tip was in direct contact with the sample and reflectance data was collected using proprietary software

(Spectra Suite, Ocean Optics). The baseline spectrum from native tissue at 0 min with that of optically cleared tissue. Percentage change in reflection spectrum due to OCA was calculated and collected every 30 s for 30 min.

2.3 Excised Vaginal Tissue Preparation

Human vaginal tissue measuring $1.5\text{ cm} \times 5\text{ cm}$ was excised. The cadaver tissue was defrosted and stored in the lab at room temperature ($\sim 20\text{ }^{\circ}\text{C}$) for 3 days. An OCA (glycerol) was applied to distal end of tissue for 30 min for the spectrum study (Figure 2A). Afterwards, tissue was rehydrated with saline for 15 min before laser treatment study. The excised vaginal tissue exhibited non-uniform coloration from distal to proximal end (Figure 2B).

2.4 Optical Coherence Tomography

Optical coherence tomography (OCT) of vaginal tissue was also performed with a clinical OCT system (Niris, Imalux, Cleveland, OH) using a handheld 8 Fr probe (2.67-mm-OD). The time-domain OCT system, based on a common-path all fiber interferometer, operated at a wavelength of 1300 nm with $15\text{ }\mu\text{m}$ axial and $25\text{ }\mu\text{m}$ lateral resolution. OCT images of vaginal tissue before and after glycerol application were acquired to determine image depth with and without OCA. Measurements were performed using software (ImageJ, NIH, Bethesda, MD) to analyze the 256-gray scale image. OCT data were recorded every 5 min during OCA experiments ($n=4$). Vaginal tissue was then excised and the transvaginal laser probe used to deliver laser energy.

2.5 Laser Probe Design and Treatment Parameters

Thermal lesions were created in vaginal tissue using a Ytterbium fiber laser with a wavelength of 1075 nm, incident power of 4.6 – 6.4 W, spot diameter of 5.2 mm, and irradiation time of 30 s. Laser energy was delivered through a custom-built probe head ($19 \times 22\text{ mm}$) with integrated pressure and temperature sensors, and flow cell circulating an alcohol-based solution for cooling to a temperature of $-2\text{ }^{\circ}\text{C}$ (Figure 3). Both non-invasive and invasive procedures were carried out in the third cadaver study. The laser probe was tested and the spectrum study from the second cadaver was repeated ($n=4$) to confirm the data.

3. RESULTS

3.1 Ultrasound Imaging Studies

In the first cadaver study, US images were obtained to locate the ideal treatment zone of the endopelvic fascia, labeled in Figure 4. The second cadaver study further confirmed the treatment zone to be at 12 o'clock and $\sim 3\text{ cm}$ deep from the vaginal opening when the patient was in the supine position. In third cadaver study, the force sensor on laser probe was tested and a 0.3 – 0.6 N force was judged to be sufficient to insure consistent contact.

3.2 Reflection Spectroscopy

Reflection percentage change data was collected every 30 s in range from 500 – 800 nm and compiled in 50 nm increments. OCA experiments using glycerol were repeated four times. Glycerol diffused deeper into the tissue and OCA effects diminished over time when vaginal tissue was intact. In Figure 5, the U part of blue curve shows that solution diffused into surrounding tissue. It also indicates that optimal treatment time was about 11 min with ~ 15% transmission change. When the vaginal tissue was excised, glycerol completely saturated the tissue and increased transmission ~ 65 %. In Figure 5, excised vaginal tissue has a reflection spectrum that decays over time.

3.3 Optical Coherence Tomography (OCT)

Since gray scale intensity is directly proportional to OPD, intensity decrease ($1/e$) is equal to a gray scale value of 94. Figure 6a shows initial OCT image at $t = 0$ min. A $1/e$ intensity decrease was measured at depth of $d = 0.464$ mm. After an optimal OCA application time of 10 min (Figure 6c), new $1/e$ gray scale value of 94 increased to a depth of $d = 0.536$ mm, translating to a 15.5% increase in OPD with OCA, which closely matches optimal treatment time of 11 min from reflection spectrum data (Figure 5). OCT image data at 1300 nm for intact vaginal tissue also has a transmission change of ~17%, consistent with spectrum transmission change at 11 min of ~ 15%.

3.4 Laser Treatment

Since human cadaver tissue was non-uniform from distal to proximal end, a power escalation study was conducted at 30 s irradiation time, with 5 min between studies. Subsurface lesions were made using 4.6 and 6.4 W. The 4.6 W laser setting preserved a deeper surface tissue layer than the 6.4 W setting (1.1 vs 0.8 mm) while also creating a larger lesion area (4.6 vs 3.1 mm²), as shown in Figure 7 and summarized in Table 1. This may be due in part to the difference in initial tissue temperature and slight non-uniformity of tissue composition from distal to proximal end.

4. DISCUSSION

It was previously reported that at 22 °C porcine vaginal tissue with glycerol treatment for 10 min yielded a ~16% transmission change at 1075 nm [12]. This data is in close agreement with the 1300 nm OCT data of ~17% transmission change after 10 min. Previous OCA data at 1075 nm also agrees with the average reflectance spectrum change of 15% at 11 min. At body temperature (37 °C), OCA treated porcine vaginal tissue transparency improved by ~2 times greater than at room temperature (22 °C) for the same treatment period of 10 min. Assuming that human and pig vaginal tissue are similar, the OCA should improve transmission by ~33% at body temperature (37 °C) after 10 min. Subsurface lesions in human vaginal tissue also demonstrated feasibility of non-invasive laser SUI treatment. However, further optimization of the laser and cooling parameters for thermal remodeling is required.

5. CONCLUSIONS

Optical studies in cadavers demonstrated subsurface laser targeting of endopelvic fascia with partial preservation of the vaginal wall, using deeply penetrating near-IR laser energy and contact cooling. OCA (glycerol) application for 10 min improved optical penetration by 15% at room temperature, with a calculated 33% increase at body temperature. Further studies will optimize laser parameters for thermal remodeling with tissue surface preservation.

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Figure 1.
(A) Ultrasound machine with (B) endovaginal probe. (C) Spectrometer and Y-cables setup for cadaver data collection.

Figure 2.
(A) Setup for reflectance spectrum response of glycerol over a period of 30 min. (B) Excised vaginal tissue.

Figure 3.
(A) Diagram of probe design.(B) Photograph of probe.

Figure 4.
(A) US image of cadaver vaginal canal; (B) Diagram for vaginal tissue layers [9,10].

Figure 5. Average reflection change of intact and excised vaginal tissue for wavelength range of 500 – 800 nm, as a function of OCA application time.

Figure 6.

OCT images of vaginal tissue clearing as a function of OCA application time. (a) $t = 0$ min, $d = 0.464$ mm; (b) $t = 5$ min, $d = 0.496$ mm; (c) $t = 10$ min, $d = 0.536$ mm; (d) $t = 15$ min, $d = 0.528$ mm; (e) $t = 20$ min, $d = 0.480$ mm; (f) $t = 25$ min, $d = 0.472$ mm; (g) $t = 30$ min, $d = 0.468$ mm.

Figure 7.

Thermal lesions produced in human vaginal tissue, ex vivo, using 1075 nm laser. (A) 4.6 W for 30 s preserved 1.1 ± 0.2 mm; (B) 6.4 W for 30 s preserved 0.78 ± 0.1 mm.

Table 1.Lesion characteristics ($\lambda = 1075$ nm).

Parameters		
Laser Incident Power (W):	4.6	6.4
Irradiation Time (s):	30	30
Initial Tissue Temp. (°C):	20	17
Probe Temp. (°C):	−2	−2
Lesion Characteristics		
Preserved Tissue Layer (mm):	1.1 ±0.2	0.8 ±0.1
Lesion Area (mm ²):	4.6 ±0.6	3.1 ±0.3
Lesion Shape:	Circular	Elliptical