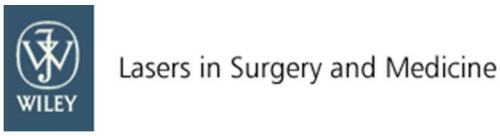

Selective Photothermolysis to Target Sebaceous Glands: Theoretical Estimation of Parameters and Preliminary Results Using a Free Electron Laser

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Complete List of Authors:	Sakamoto, Fernanda; Wellman Center for Photomedicine - Massachusetts General Hospital - Harvard Medical School, Dermatology Doukas, Apostolos; Wellman Center for Photomedicine - Massachusetts General Hospital - Harvard Medical School, Dermatology Farinelli, William; Wellman Center for Photomedicine - Massachusetts General Hospital - Harvard Medical School, Dermatology Tannous, Zeina; Wellman Center for Photomedicine - Massachusetts General Hospital - Harvard Medical School, Dermatology Shinn, Michelle; Thomas Jefferson National Accelarator Facility Benson, Steve; Thomas Jefferson National Accelarator Facility Williams, Gwyn; Thomas Jefferson National Accelarator Facility Gubeli III, Joseph; Thomas Jefferson National Accelarator Facility Dylla, H. Frederick; American Institute of Physics Anderson, R. Rox; Wellman Center for Photomedicine, Massachusetts General Hospital
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Selective Photothermolysis to Target Sebaceous Glands: Theoretical Estimation of Parameters and Preliminary Results Using a Free Electron Laser.

Fernanda H. Sakamoto, M.D., Ph.D. (1,2), Apostolos G. Doukas, Ph.D. (1,2), William A. Farinelli, B.A. (1,2), Zeina Tannous, M.D. (1,2), Michelle Shinn, Ph.D. (3), Steve Benson, Ph.D. (3), Gwyn P. Williams, Ph.D. (3), Joseph F. Gubeli III, M.S. (3), H. Frederick Dylla, Ph.D. (4), R. Rox Anderson, M.D. (1,2).

1. Wellman Center for Photomedicine, Department of Dermatology, Massachusetts General Hospital, Boston, MA, USA, 02114.
2. Harvard Medical School, Department of Dermatology, Boston, MA, USA, 02114
3. Thomas Jefferson National Accelerator Facility, Newport News, VA, USA, 23606.
4. American Institute of Physics, College Park, MD 20740.

Corresponding author: R. Rox Anderson, MD.

55 Fruit St. BHX 630

02114 Boston, MA, USA

Phone: +1-617-7266168 Fax: +1-617-7266121

E-mail: rranderson@partners.org

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Abstract.

Background and Objectives: The success of permanent laser hair removal suggests that selective photothermolysis (SP) of sebaceous glands, another part of hair follicles, may also have merit. About 30% of sebum consists of fats with copious CH_2 bond content. SP was studied in vitro, using free electron laser (FEL) pulses at an infrared CH_2 vibrational absorption wavelength band.

Methods: Absorption spectra of natural and artificially prepared sebum were measured from 200 nm to 3000 nm, to determine wavelengths potentially able to target sebaceous glands. The Jefferson National Accelerator superconducting FEL was used to measure photothermal excitation of aqueous gels, artificial sebum, pig skin, human scalp and forehead skin (sebaceous sites). *In vitro* skin samples were exposed to FEL pulses from 1620 to 1720 nm, spot diameter 7-9.5 mm with exposure through a cold 4^{o}C sapphire window in contact with the skin. Exposed and control tissue samples were stained using H&E, and nitroblue tetrazolium chloride staining (NBTC) was used to detect thermal denaturation.

Results: Natural and artificial sebum both had absorption peaks near 1210, 1728, 1760, 2306 and 2346 nm. Laser-induced heating of artificial sebum was approximately twice that of water at 1710 and 1720 nm, and about 1.5x higher in human sebaceous glands than in water. Thermal camera imaging showed transient focal heating near sebaceous hair follicles. Histologically, skin samples exposed to ~1700 nm, ~100-125 ms pulses showed evidence of selective thermal damage to sebaceous glands. Sebaceous glands were positive for NBTC staining, without evidence of selective loss in samples exposed to the laser. Epidermis was undamaged in all samples.

Conclusions: SP of sebaceous glands appears to be feasible. Potentially, optical pulses at ~1720 nm or ~1210 nm delivered with large beam diameter and appropriate skin cooling in approximately 0.1 s may provide an alternative treatment for acne.

Introduction

Selective Photothermolysis (SP) has been used to treat many different disorders including glaucoma, gastrointestinal lesions, vascular malformations, vocal cord lesions, verruca, pigmented lesions, tattoos, and hair follicles (1). In general, specific chromophores of the tissue are necessary (such as hemoglobin, melanin, etc) for SP to selectively deposit energy in or near the target. Appropriate source wavelength, beam optics, and pulse duration nearly equal to the thermal relaxation time of the desired target structure are generally required (2,3). Static or dynamic tissue cooling can be applied to protect adjacent tissues from thermal injury (4).

Acne is the most common skin disease and the most common cause of permanent facial scarring (5-7). For the treatment of acne, lasers have been used with wavelengths of 1450 nm (8) (9), 585-595 nm (PDL, pulsed dye lasers) (10-13), and 535 nm (KTP) (14). None of these wavelengths are preferentially absorbed by sebaceous glands, but may improve acne to some extent. Statistically significant reduction of lesion count and severity with histological thermal damage in the upper dermis was observed using 1450 nm (8). This wavelength is well absorbed by water, inducing an improvement effect in acne by unknown mechanisms. Mechanism of action and efficacy using PDL and KTP lasers are controversial (11-13), and may involve SP of microvessels. No studies of laser or light treatments have shown convincing evidence to improve non-inflammatory acne. To date, laser treatment of acne is limited, expensive and requires multiple sessions.

All medical applications of SP at present are based on electronic absorption transitions; however there is no intrinsic limitation to using vibrational or other excitation modes. Anderson et al (15) first described the use of CH_2 bond vibrational absorption modes as

a means for SP of lipid-rich tissues, identifying two absorption bands near 1210 and 1720 nm. At both wavebands, lipids have somewhat stronger optical absorption than water, but the contrast is weak. Of these wavelengths, 1210 nm has deeper tissue penetration. It was shown that 1210 nm exposures are capable of preferentially damaging subcutaneous fat when delivered in combination with skin cooling. Subcutaneous fat is a thick layer that retains heat well; such that even very long exposures (20 seconds) allow thermal confinement in subcutaneous fat. In contrast, sebaceous glands are typically small intradermal structures (diameters 100-500 μm, average 250 μm) (16), with an estimated thermal relaxation time of about 100 – 250 milliseconds or less. While the 1210 nm wavelength band is well suited for targeting subcutaneous fat due to deeper penetration, in theory, both the 1210 nm and 1720 nm wavelength bands could be used for targeting sebaceous glands.

Sebum produced by sebaceous glands could possibly be used as a chromophore for SP. About 30% of sebum is composed of free fats, triglycerides, squalene, cholesterol and wax esters (17) with copious CH_2 bond content. In human tissue, sebaceous glands are located about 1 mm deep (18). In this pilot study, we studied optical absorption spectra of sebum, measured the wavelength-dependant photothermal excitation of sebum, water and tissue samples, and looked for histological evidence of sebaceous gland SP with optical pulses near 1700 nm.

Materials and Methods

Spectrophotometry

A Cary 5000 integrating sphere spectrophotometer (Varian Inc., Walnut Creek, CA, USA) was used for measurements of absorption spectra of natural and artificial sebum and for transmittance/reflectance measurements of human skin from 400 to 2500 nm.

Human skin spectroscopy

Human skin - Measurements of total spectral transmittance through human skin were performed using a slightly modified integrating sphere spectrophotometer, conducted on discarded tissue collected from abdominoplasty and face-lifting surgery of three Fitzpatrick skin phototype I-III patients performed at Massachusetts General Hospital after Institutional Review Board approval. Fresh frozen tissue samples 3 x 2 cm were thawed and subcutaneous fat was removed leaving approximately 2 mm-thick samples, containing full thickness of epidermis and dermis. Tissue was placed on a microscope cover slip (0.17 mm thick, 35 x 60 mm, Gold Seal® Cover Glass, ©2008 Thermo Fisher Scientific Inc. Portsmouth, NH, USA) with epidermis facing the spectrophotometer light beam, at the entrance port of the integrating sphere. Skin transmittance and reflectance were measured every 2 nm from 400-2500 nm in a single-beam measurement mode, using the microscope cover slip and lens assembly alone as reference.

Absorption spectra of sebum

Natural Sebum (NS) - Human sebum from 10 male adults' noses and foreheads was collected on microscope slides 1 hour after the skin was cleansed with water, soap and thorough rinsing. Ages varied from 25 to 64 years old (median 35 years old). The material collected was dissolved in carbon tetrachloride (CCl_4) (Sigma-Aldrich, Saint

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Louis, MO, USA), and studied by absorption spectrophotometry set to measure every 2 nm from 200-3000 nm, using CCl_4 as reference. This yielded qualitative absorption spectra in the near and mid-infrared region for human sebum.

Artificial Sebum (AS) – A mix of artificial sebum lipids was prepared according to Nordstrom et al.'s report of sebum content (17) using 4% cholesterol (Sigma-Aldrich, Saint Louis, MO, USA), 25% wax ester (palmitic acid stearyl ester, Sigma-Aldrich, Saint Louis, MO, USA), 20% squalene (Sigma-Aldrich, Saint Louis, MO, USA), 16% triglycerides (tripalmitin, Sigma-Aldrich, Saint Louis, MO, USA) and 33% free-fatty acid (palmitic acid free-acid, Sigma-Aldrich, Saint Louis, MO, USA). The artificial sebum lipid was a translucent material that was dissolved in CCl_4 to obtain a transparent solution for absorption spectrophotometry. A total of 0.4 g of AS was diluted into 1 mL of CCl_4, measured in a 1 mm cuvette against CCl_4 as the reference.

Monte Carlo modeling

A Monte Carlo model of light interaction with tissue was used (19), to analyze the spectrophotometry measurements of ex vivo skin. The input data was total transmittance (Tt), collimated transmittance (Tc), diffuse reflectance (Rd) and skin sample thickness. The *Henyey-Greestein* scattering phase function with an average cosine, *g*, was set to 0.9 (20). Refractive index, *n,* of tissue was set to be 1.4 (21). Simulation was performed at 1210 nm and 1720 at 1.0 mm and 2.0 mm deep, corresponding to estimated SG and fat depth of location, using the inverse adding-doubling (IAD) method (22). Absorption coefficient μ_a and scattering coefficient $\mu_{s'}$ of skin were, then, calculated.

Tissue and other samples exposed to the FEL

An aqueous gel was prepared using 6% (w/w) gelatin (Knox, Kraft Foods, Tarrytown, NY, USA), to represent water (94% water, 6% collagen).

In humans, face and scalp are rich in sebaceous glands. Full thickness forehead and scalp skin obtained from face-lifting plastic surgery, and adult porcine (Yorkshire) flank skin obtained at euthanasia were used as samples. Tissue for the experiment was fresh frozen, and thawed prior to laser exposure. The dimensions of sebaceous glands from these human facial skin samples were measured using light microscopy (H&E stained slides), and average sizes used to estimate the thermal relaxation time of human adult facial sebaceous glands.

In some samples used for imaging and measurement of photothermal excitation of sebaceous glands *in situ*, the epidermis and upper dermis were removed by horizontal excision with a dermatome to a depth of ~0.5-1.0 mm to expose the region of dermis where most of the sebaceous glands are located.

Free Electron Laser (FEL)

The superconducting electron accelerator-driven FEL from the Thomas Jefferson National Accelerator Laboratory was adjusted to allow wavelength tuning and broad output power range up to 1000W. The FEL produces a train of 500-1700 femtosecond pulses (FWHM), which in accordance with Heisenberg's principle produces a broad wavelength bandwidth that typically varied from 15-25 nm (23,24). Spectrometer scans were obtained at each wavelength. The set up and bandwidth were similar to that reported by Anderson et al. (15), but dielectric mirrors were used in the laser cavity in

order to tune laser wavelengths from 1620 to 1720 nm, and metal mirrors were used from 1600 to 1800 nm. FEL beam diameter at the sample was adjusted with lenses to a range of 7.0-9.5 mm round spots. An aperture was used to improve beam uniformity by removing the edges of the Gaussian beam profile. The estimated incident exposure beam uniformity on samples was ± 30%. FEL exposure duration was controlled at the accelerator level, by adjusting the electron source duration from 25-250 ms.

Photothermal excitation measurements

FEL-induced temperature rise was measured at the surface of human tissue samples and other samples (artificial sebum lipids, aqueous gels) by photothermal radiometry using a 3~5 μm thermal camera (PM180 Thermacan, FLIR Systems, Inc., N. Billerica, MA, USA) calibrated for the experiment, during FEL exposure of the samples. Camera software includes peak-temperature detection at a given location in the field of view, which was aligned with the center of the FEL beam incident upon a sample's surface. The thermal camera was blind to the FEL wavelengths. The maximum temperature was limited to less than 65^{o} C to minimize tissue thermal damage for this part of the experiment. Photothermal excitation was normalized for beam energy by dividing each exposure's induced peak temperature rise of the sample (ΔT), by the associated FEL exposure fluence (E) (result expressed in $^{o}C{\cdot}J^{-1}{\cdot}cm^{2}$). The mean and standard deviation (SD) of 6 independent measurements of the photothermal excitation were determined for each sample at each wavelength.

Statistical analysis of photoexcitation measurements was performed using SPSS Version 15. (SPSS Inc., Chicago, IL, USA), and Microcal™ Origin® Version 6.0 (Microcal Software Inc., Northampton, MA, USA).

Study design

Initially, the photothermal excitations for human facial skin, pig skin, artificial sebum and water were determined for wavelengths from 1620 nm to 1720 nm (increasing 20 nm per exposure) at 25 ms pulse duration. Six measurements for each wavelength were taken for statistical validation. An optimal wavelength region was defined as the wavelength which produced statistically significant greater thermal rise for the sebaceous glands/sebum samples over water gels/dermis samples.

In the second part of the experiment, human and porcine skin samples were exposed to FEL pulses ranging systematically from 100-185 milliseconds (ms) at the ~ 1700 nm wavelength determined from photothermal excitation measurements to be promising for SP of sebaceous glands. Human skin samples were either prepared with their top removed to expose the glands as described, or intact. For intact skin samples, the dermal side was placed on a warm (37^{o} C) plate, and the FEL was delivered through a cold sapphire window pressed onto the epidermal surface, as previously described (15) Briefly, the sapphire window (3 mm thickness x 50 mm diameter) was mounted in thermal contact with a copper block through which ice water was passed by means of a small pump, maintaining the window temperature of 6-8 ^{o}C.

The cold contact sapphire window was used to protect overlying epidermis and superficial dermis during exposures. Samples were temperature equilibrated for at least 30 seconds prior to each FEL exposure. At least 6 samples were exposed to each of a range of FEL fluences at each wavelength and pulse duration, to allow histological examination of independent, repeated samples.

The measured fluence delivered to samples ranged from 64 to 120 J/cm^2. The precision of pulse-to-pulse fluence measurement was approximately ± 5%. However, the absolute calibration error of the absolute FEL incident fluence was estimated to be up to ±50% due to the combined uncertainties of energy measurement, beam diameter and beam profile variations in the exposure area.

Tissue Thermal Effects Assessment

Exposed and unexposed control tissue samples were bisected with a razor blade through the optical exposure axis. One half was processed for routine light microscopy using hematoxylin and eosin staining (H&E) and for polarized light microscopy. Loss of birefringence is a marker of type I collagen thermal damage when denaturation occurs at 70 ^{o}C. (25). The other half was incubated for 15 minutes at room temperature in a solution containing nitroblue tetrazolium chloride (NBTC) according to Neumann's technique for visualizing activity of NADPH diaphorase, a thermally sensitive enzyme (26). Cell lethality caused by thermal denaturation is well correlated with loss of enzyme activity, and with consequent loss of NBTC dark blue staining.

Histological evidence of tissue changes was blindly assessed by a dermatopathologist, who compared FEL exposed with unexposed control sites.

Results

Absorption spectra

Quantitative absorption spectra are shown in figure 1, for artificial sebum and water. Natural sebum showed weak absorption maxima at 1212 nm and 1364 nm, stronger maxima at ~ 1720 and 1760 nm, and strong absorption at 2306 and 2346 nm. Due to the

very small amount of sebum collected, a quantitative spectrum was not possible for human sebum. Absorption spectra of artificial sebum was qualitatively very similar to natural sebum, with absorption maxima at 1210 nm; 1390 and 1414 nm; 1728 and 1760; 2306 and 2346 nm. At 1210 nm, artificial sebum lipid absorption was measured to be nearly equal to that of water (figure 1); while artificial sebum lipids had higher absorption than water at 1726 nm, 2306 nm and at 2346 nm.

When optical absorption by the 70% water content and 30% lipids content of sebum *in vivo* is considered (17), the resultant spectrum shown in figure 1 is obtained. At 1726 nm, *in vivo* sebum is estimated to have 1.20 times the absorption of water.

Size of human sebaceous glands

The length and width of human facial skin sebaceous glands were 0.26 mm (SD ± 0.11), and 0.12 (SD ± 0.12) mm respectively. Facial sebaceous glands were found from 0.12 to 0.76 mm deep from the surface of the skin; average of 0.50 mm (SD ± 0.19).

Monte Carlo modeling

At 1200 nm, calculated $\mu_a = 1.22\ (\pm 0.21)\ \text{cm}^{-1}$ and $\mu_{s'} = 14.45\ (\pm 2.24)\ \text{cm}^{-1}$. At 1720 nm, calculated $\mu_a = 9.63(\pm 2.02)\ \text{cm}^{-1}$ and $\mu_{s'} = 9.78\ (\pm 1.46)\ \text{cm}^{-1}$. Table 1 summarizes results of Monte Carlo simulation.

Estimation of exposure parameters to target sebaceous glands

The local temperature rise (ΔT) within tissue from absorption of a short optical pulse, ignoring heat diffusion during the pulse, can be estimated as $\Delta T = \mu_a F_o T/(\rho c)$ where μ_a is

the local optical absorption coefficient (typically in units of cm^{-1}), F_o is the incident optical fluence at the tissue surface (typically in units of J/cm^2), *T* is the effective optical transmittance through tissue to the site of interest, ρ is density and c is specific heat capacity. The density and heat capacity of water-rich tissues such as epidermis and dermis are very close to those of water, while density and heat capacity of lipid-rich tissues such as fat and sebaceous glands are lower. For dermis, $c \approx 4.2\ J.g^{-1}K^{-1}$ and $\rho \approx 1\ g/cm^3$. For fat, $\rho c \approx 2.1\ JK^{-1}cm^{-3}$, half that of dermis (27). For sebaceous glands with 30% lipid content (17), ρc is estimated to be approximately $3.6\ JK^{-1}cm^{-3}$.

The optimal pulse duration for SP is typically equal to or less than the thermal relaxation time (τ) of the target structure, in this case sebaceous glands (2). In units of seconds and millimeters for biological targets, $\tau \approx diameter^2$. According to our measurements of human facial sebaceous glands in this study, their thermal relaxation time ranges from about 0.020 to 0.129 seconds, with a mean of about 0.06 s. In theory, selective photothermolysis of sebaceous glands would be expected from appropriate optical pulses of about 0.1 second or 100 ms.

To estimate the incident fluence (F_o) expected for SP, the temperature rise (ΔT) should be estimated. The baseline temperature (T_{SG}) at the SG level (~0.5-1.0 mm depth) and subcutaneous fat (~2.0 mm depth) while a cold window is applied for epidermal protection at 4^oC was estimated, we considered Fourier's law integrated for a simple exponential situation, where uniform temperature across equally sized end surfaces and perfectly insulated sides exist, and the heat flow rate between the end surfaces is given by:

$$\frac{\Delta Q}{\Delta t} = k\ A\ \frac{\Delta T}{\Delta x}$$

Where $\Delta Q/\Delta t$ is the heat flow by a given time period, k is the material conductivity, A is the cross-sectional surface area, ΔT is the temperature difference between the ends, and Δx is the distance between the ends.

When cooling is applied in a large enough area until an equilibrium state, it behaves as a two dimension model, where: T_{surf}, is the temperature at the surface (cooling plate at 4°C), T_b is the baseline body temperature at the subcutaneous fat level (37°C), T_{SG} is the baseline temperature at the SG level, x_b is the distance from the surface to the top of subcutaneous fat (2 mm), x_{SG} is the distance from the surface to the SG. From the simple exponential Fouries's law:

$$T_{SG} = \frac{x_{SG}}{x_b}\left(T_b - T_{surf}\right) + T_{surf}$$

Using the simplified heat equation, the estimated baseline temperature at the sebaceous glands inside the tissue samples of this study was approximately 12°C.

For most mammalian cells such as SG cells heated for 0.1-1 second, cell lethality is achieved at temperatures of about 55-65°C. The temperature rise (ΔT_{SG}) expected for thermal damage to SG in this study is therefore about 50°C.

Parameters for the demonstrated and theoretical selective photothermolysis of lipid rich tissues are summarized in table 1.

FEL Photothermal Excitation

Photothermal excitation measurements are summarized in figure 2. At fixed pulse duration (25 ms), the three different samples (human sebaceous glands, AS and water)

showed statistically different photothermal excitation ($p < .05$, Tukey and Scheffe tests). Laser induced transient heating of the artificial sebum was approximately 2.3x that of the aqueous gel at 1700 nm, and about 1.4x higher in human sebaceous glands *in situ* than in the aqueous gel. At 1720, nm heating of artificial sebum was 2.1x higher than aqueous gel and 1.6x higher in human sebaceous glands *in situ* than the gel.

As a preferential photothermal excitation of artificial sebum and human sebaceous glands was produced at about 1700-1720 nm, we measured sebaceous glands photothermal excitation with different pulse widths at an FEL wavelength of ~1700 nm.

Thermal camera images of the samples sectioned horizontally ~ 1 mm deep showed preferential thermal excitation in small dots corresponding to follicles when exposed to FEL at 1700-1720 nm. When wavelength was fixed at 1700 nm, "hot-spots" were apparent and obvious near hair follicles at the surface of tissue for pulse width up to 100 – 125 ms. At 1700 nm, the contrast between of photothermal excitation of sebaceous glands, with that of adjacent tissue, decreased for exposures > 100 ms.

Microscopy: NBTC Staining

NBTC staining in unexposed control samples was strong and variable in sebaceous follicles, which made it difficult to assess changes in SG staining in the exposed samples. There was non-selective or complete loss of dermal NBTC staining after FEL exposure (1700 nm) at pulse widths higher than and equal to 135 ms, corresponding to FEL fluence 87.6 to 120 J/cm^2. At 100 and 125 ms, weak NBTC staining could be observed in the epidermis and dermis at the laser exposed sites, but it was difficult to determine if there was decreased staining in sebaceous glands (figure 3).

Microscopy: H&E Staining

Blind assessment of H&E-stained histopathology sections of FEL-exposed full thickness human sebaceous skin showed no changes in the epidermis and upper dermis for FEL exposures up to ~ 120 J/ cm^2 delivered in 175 ms, at and above which there was subepidermal blister formation consistent with thermal injury (Figure 4A). At FEL exposures of 100 and 125 ms (64-70 J/cm^2 at 100 ms, and 78-82 J/cm^2 at 125 ms) no epidermal or superficial dermal injury was seen. At these exposures, histological changes were limited to sebaceous glands and sebaceous follicles. There was thickening and altered staining of collagen bundles around the sebaceous follicles, loss of sebocytes structural arrangement and nuclear changes consistent with thermal injury to pilosebaceous units (PSU) (Figure 5B and 5C). Samples that were exposed to pulses longer than and equal to 135 ms (87.6-93.5 J/cm^2) showed extensive non-selective thermal denaturation of dermis (Figure 5D).

Loss of birefringence is a marker of thermal damage to dermal collagen. Reduction of collagen birefringence was observed after 1700 nm FEL exposures longer than or equal to 135 ms (Figure 5B).

Discussion

The primary new findings of this study are: (1) the determination of optical absorption spectra for sebum in the near infrared; (2) determination of optimal wavelengths for selective photothermolysis to target sebaceous glands, at approximately 1210 nm, 1726 nm and 1760 nm [the strong 2306 and 2346 nm absorption band wavelengths do not

penetrate to the depth SG *in vivo*] ; (3) estimation of human facial sebaceous glands thermal relaxation time to be about 100 ms; (4) estimation of the parameters for targeting SG using about 50 J/cm^2 incident fluence at 1720 nm; and (5) preliminary evidence by H&E stained histology of selective photothermal targeting of sebaceous glands using an FEL at 1700 – 1720 nm, with optical pulse widths from 100 to 125 ms and fluences of 65-82 J/cm^2 delivered with skin cooling to protect the epidermis. These experimental findings are consistent with theoretical values (table 1). However, theory also predicts a relatively narrow range of parameters to achieve SP of sebaceous glands, compared with other known examples of SP.

Anderson et al. (2006) previously reported absorption spectra for subcutaneous fat, and demonstrated photothermal damage of subcutaneous fat using the Jefferson Laboratory FEL near 1200 nm, but the possibility of targeting sebaceous glands was not investigated. Sebum lipids are markedly different from those of fat, and the absorption spectra of sebum in the infrared reported here, are novel. We also demonstrated that natural and artificial sebum have similar absorption spectra. Near infrared absorption by lipids is due to vibrational overtone modes, which are combination frequencies of the fundamental modes found in the mid infrared region (28). Interestingly, both natural and artificial sebum have interesting double peaks that are related to different harmonic vibrations of the same chemical bonds. The absorption bands at wavelengths over 2200 nm are in the region of C-H combination bands that could correspond to CH+CH, CH-CC, CH_2 or CH_3 (28). At about 1700 nm is the 1st overtone C-H stretching region (with two peaks representing, for example, CH and CH_2) (28). The peaks at about 1400 nm represent 1st CH combination overtone modes that could be, for example, CH_2 and maybe CH_3 (28)).

At about 1200 nm, the 2nd overtone C-H stretching region, could represent CH vibration (28). Like fat, both artificial sebum and natural sebum presented peaks near 1210 nm and at about 1726 nm and 1760 nm. At these regions, the absorption coefficient of sebum is higher than water (Figure 1).

We used the absorption spectrophotometric data with established models of SP to estimate and to compare with thermal excitation as defined by the mean temperature rise (measured by the thermal camera) per incident J/cm^2, in a manner similar to that reported by Anderson et al (2006) (15).

In theory, selective photothermolysis of sebaceous glands will require optical pulses of about 0.1 second or less using about 245 J/cm^2 at 1210 nm and about 67 J/cm^2 at 1720 nm, when delivered with contact cooling at 4^{o}C, in a large-diameter optical beam.

The theoretical estimated values correlated well with results obtained in this pilot study, but an exact comparison is lacking due to experimental and biological variability. It is apparent from both, that there is a narrow range of exposure parameters for which preferential damage of SG is expected. At around 100-125 ms and 60-80 J/cm^2 incident fluence, both H&E and NBTC stained histopathology samples showed selective thermal damage around sebaceous glands, with vacuolization and disruption of the integrity of the glands. Longer pulses at higher fluences showed non-selective dermal damage, consistent with SP theory. Loss of dermal collagen birefringence is a relatively insensitive marker of dermal injury; even with FEL exposures causing extensive dermal injury by both NBTC and H&E staining, collagen birefringence was still positive.

It is to be noted that skin samples used for the FEL photoexcitation were collected from scalp and face lifting, which is a surgical procedure usually performed in aged skin; and

therefore not the ideal samples to study acne skin. Facial skin in adolescents and young adults usually presents SG twice the size of aged skin, which would probably increase the range of pulse duration for SP of sebaceous glands in young adults.

These encouraging preliminary results demonstrate the feasibility of 1700-1720 nm optical pulses to affect sebaceous glands with apparently minimal injury to other skin structures. In theory and in our experiment, the ideal pulse duration is probably about 100 ms, skin cooling is necessary to protect the overlying epidermis and upper dermis, a fluence of greater than 65 J/cm^2 is needed, and there is a narrow fluence range capable of producing selective damage to SG. It should be noted that a source at 1726 nm with bandwidth narrower than the FEL used in this study maybe superior.

Alexander et al. (29) recently reported a failed attempt to produce SP of sebaceous glands using a Raman-shifted fiber laser at 1708 nm, in vitro. Unfortunately, the conditions studied were far outside the range of appropriate parameters. A narrow beam, at only 2 mm diameter was used, which severely limits the available fluence at the sebaceous glands and favors unwanted injury to the epidermis. The available laser power was merely 4 W, compared with our study using up to 1000 W from the FEL. The exposure duration studied by Alexander et al. was ten times too long. The reported laser wavelength of 1708 nm is not ideal for SG targeting and the laser bandwidth was not reported. Finally, the authors presented a data figure obtained from our previous work (15,30) in which they apparently assume that SG lipid absorption is the same as that of subcutaneous fat. Our work here, presents the first detailed absorption spectrum for sebum lipids, which is substantially different from that of fat.

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It remains unclear whether laser pulses near 1720 nm or 1200 nm can be configured for safe and effective treatment of acne. This study at least suggests that it is possible.

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Legends

Figure 1. Measured absorption coefficients (μ_a) in cm^{-1} of artificial sebum (AS) and water For AS the 28% (w/w) solution and 1 mm pathlength cuvette used, μ_a = 23 O.D./ 0.25, where O.D. is the measured optical density.

Figure 2. Photothermal excitation of human facial skin, water and artificial sebum at different wavelengths in the infrared spectrum.

Figure 3. Human skin frozen section obtained with NBTC after 1700 nm FEL exposure at 125 ms pulse duration. Left side was exposed to the laser beam, showing weaker NBTC blue staining consistent with thermal damage after laser irradiation. Sebaceous glands stain strongly with NBTC compared to epidermis; there was no clear evidence of selective sebaceous gland injury by NBTC stain.
Ep: epidermis; PSU: pilosebaceous unit.

Figure 4. H&E slide of human skin (4x) after FEL exposure at 1700 nm, 175 ms, 117 J/cm^2. (A) Laser exposure is on the right side of the slide, showing non-selective thickening and homogenization of collagen bundles consistent with non-selective thermal damage of dermis, and subepidermal blister. (B) Although complete loss of birefringence was not observed, some reduction on the irradiated area after polarized microscopy could correspond to thermal damage of collagen type I.

Figure 5. H&E slides at 10x. (A) control; (B) 1700 nm laser exposed sites at 100 ms, (C) 125 ms and (D) 135 ms. Thicken and hyperchromatic collagen bundles around hair

follicles (HF) and loss of structure of sebocytes are consistent with selective photothermolysis of sebaceous glands (arrows). There was no evidence of epidermal or superficial dermal injury.

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Disclosure of Proprietary Interests

None of the authors have a conflict of interest regarding this publication.

Table 1. Estimated exposure parameters for selective photothermolysis of lipids structures.

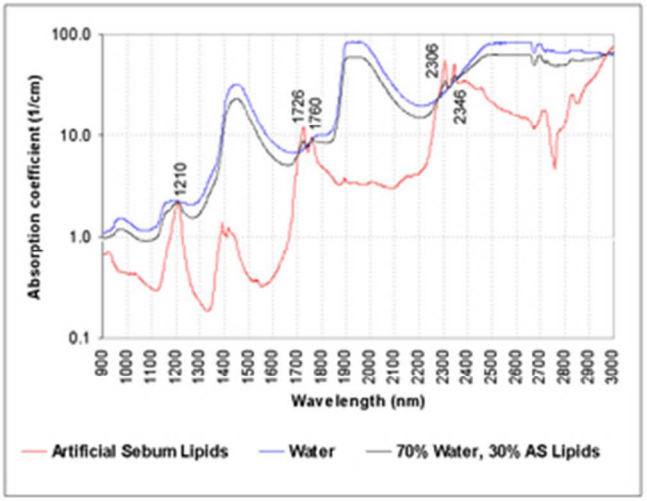
	Subcutaneous fat	Sebaceous glands
Size (mm)	> 5 (typically)	0.26 ± 0.11*
Thermal relaxation time (τ)(seconds)	> 20 (typically)	~ 0.06 – 0.100*
Depth of location (mm)	~ 2.0 (typically)	0.5 ± 0.2
ρc ($J.cm^{-3}.^oC^{-1}$)	~ 2.1 [27]	~ 3.57
Temperature raise (ΔT) (^oC)	20	50
~1210 nm band		
μ_a (cm^{-1})	1.52 [15]	2.2*
Transmittance to target (*T*)	~15.6% §	~33.1%§
Estimated fluence (J/cm^2)	~120 [15]~177 **	~ 245 **
~1720 nm band		
μ_a (cm^{-1})	10.32 [15]	8.1*
Transmittance to target (*T*)	11%§	32.8%§
Estimated fluence (J/cm^2)	~ 37 **	~ 67 **

μ_a: absorption coefficient.

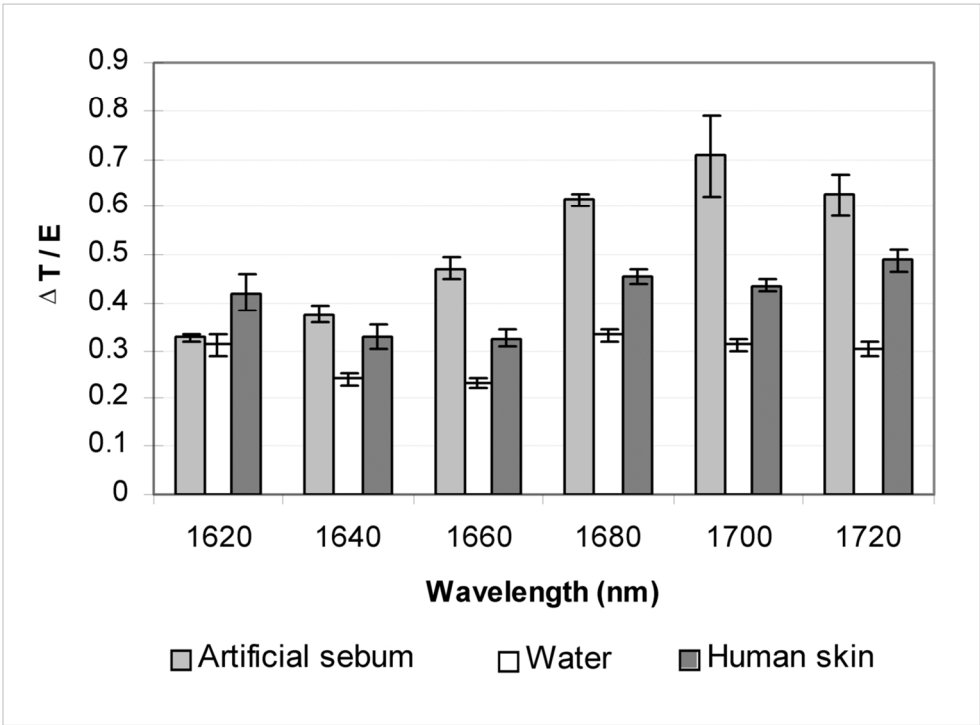
* Data from this study.

** Estimated incident fluence (F_o), $F_o \approx \Delta T.\rho c/ \mu_a.T$; where ΔT: temperature raise, ρc: heat capacity, μ_a: coefficient of absorption, *T*: transmittance through the skin.

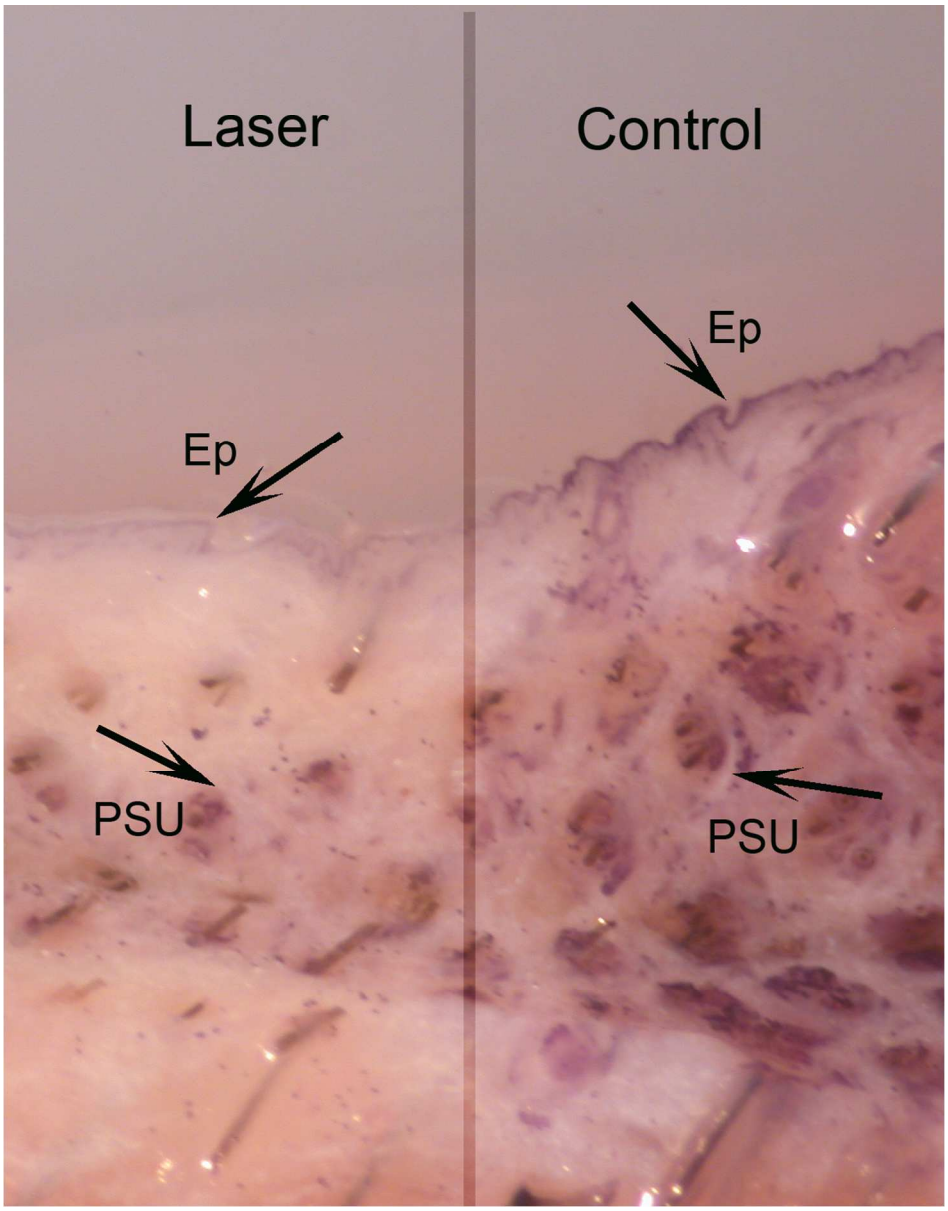
§ Estimated using Monte Carlo and IAD models.



Measured absorption coefficients (μa) in cm-1 of artificial sebum (AS) and water For AS the 28% (w/w) solution and 1 mm pathlength cuvette used, μa = 23 O.D./ 0.25, where O.D. is the measured optical density.
27x21mm (300 x 300 DPI)

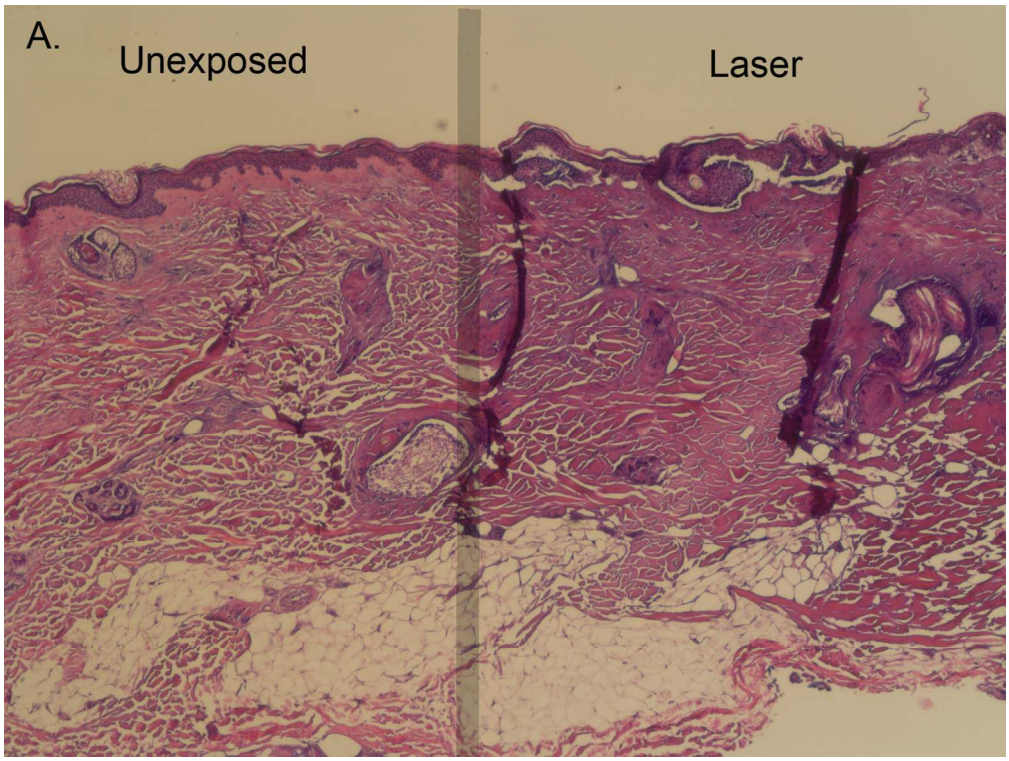


Photothermal excitation of human facial skin, water and artificial sebum at different wavelengths in the infrared spectrum.
122x90mm (300 x 300 DPI)

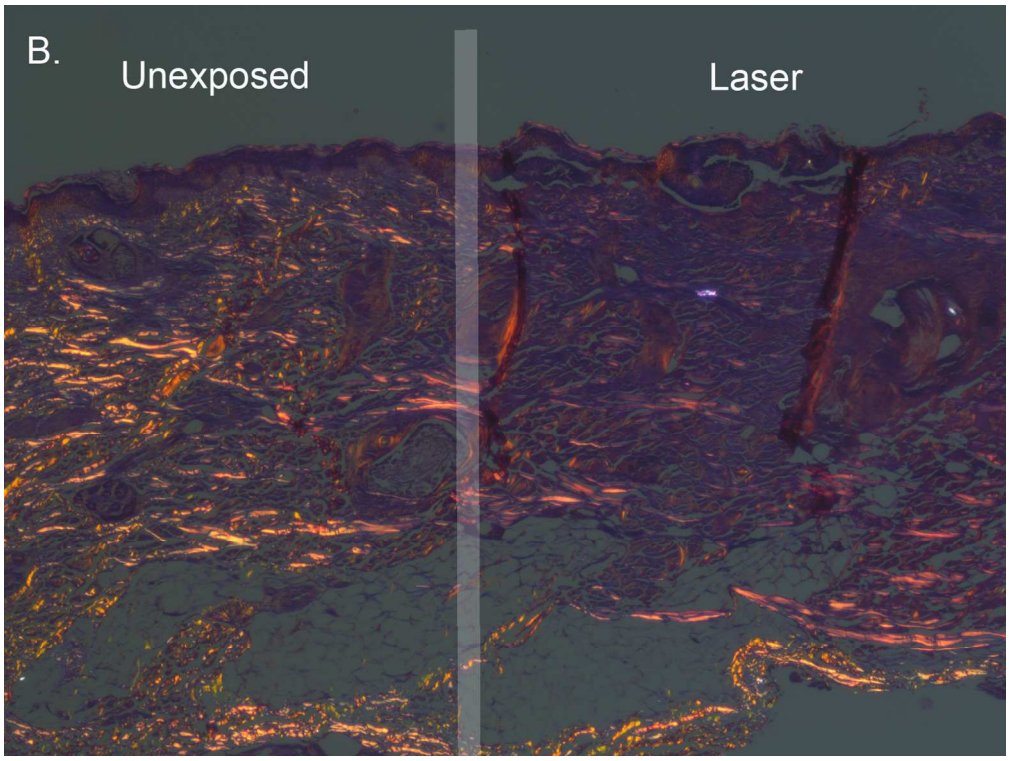


Human skin frozen section obtained with NBTC after 1700 nm FEL exposure at 125 ms pulse duration. Left side was exposed to the laser beam, showing weaker NBTC blue staining consistent with thermal damage after laser irradiation. Sebaceous glands stain strongly with NBTC compared to epidermis; there was no clear evidence of selective sebaceous gland injury by NBTC stain.
Ep: epidermis; PSU: pilosebaceous unit.

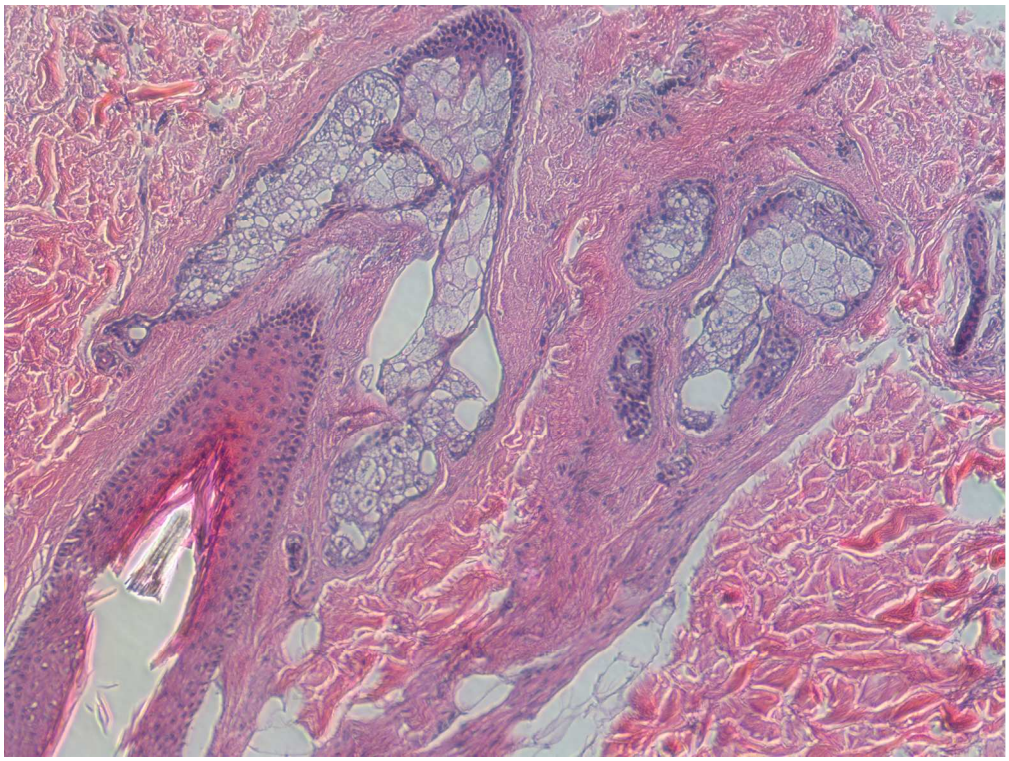
101x130mm (300 x 300 DPI)



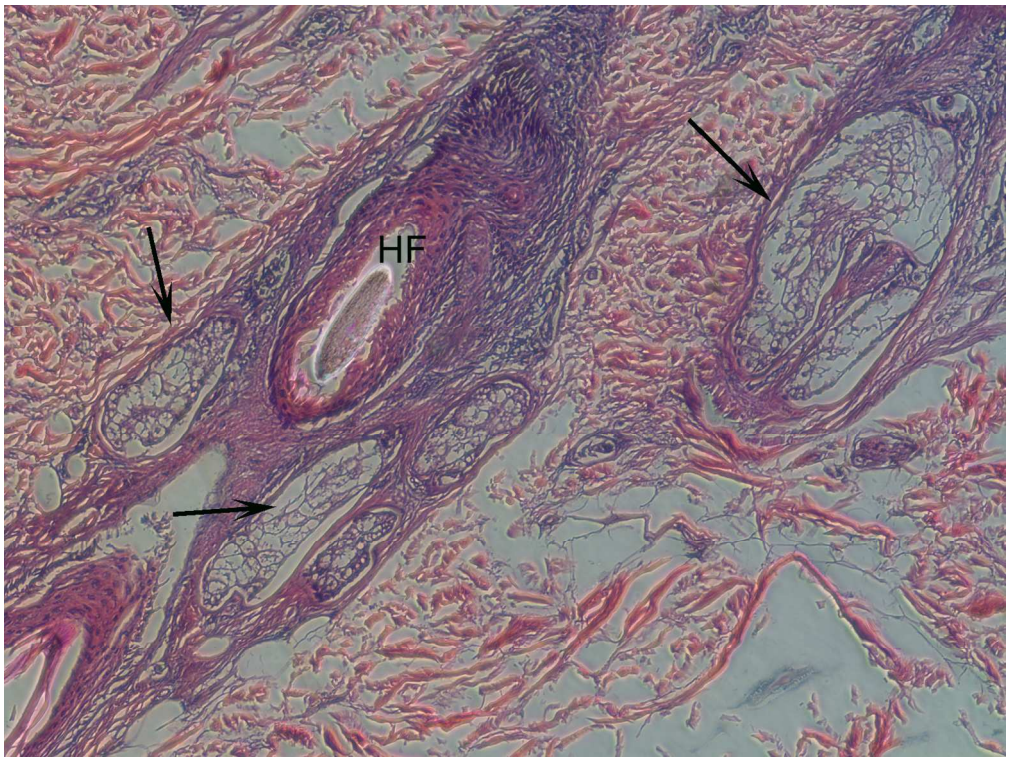
H&E slide of human skin (4x) after FEL exposure at 1700 nm, 175 ms, 117 J/cm2. (A) Laser exposure is on the right side of the slide, showing non-selective thickening and homogenization of collagen bundles consistent with non-selective thermal damage of dermis, and subepidermal blister.
564x423mm (72 x 72 DPI)



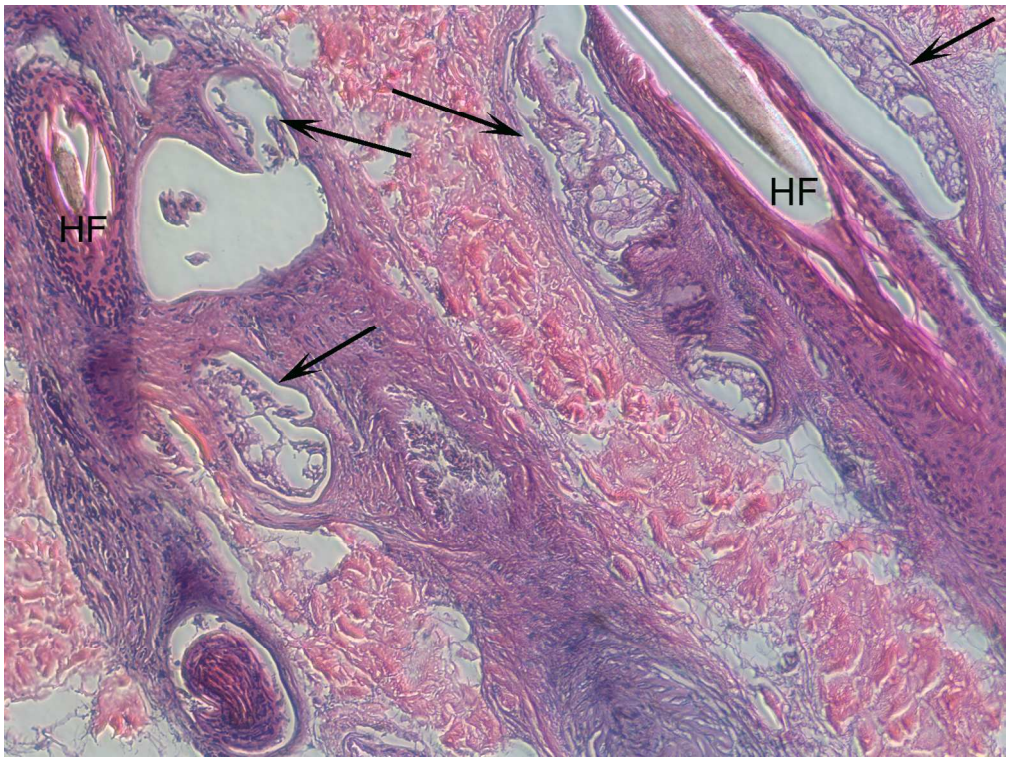
(B) Although complete loss of birefringence was not observed, some reduction on the irradiated area after polarized microscopy could correspond to thermal damage of collagen type I.
564x423mm (72 x 72 DPI)



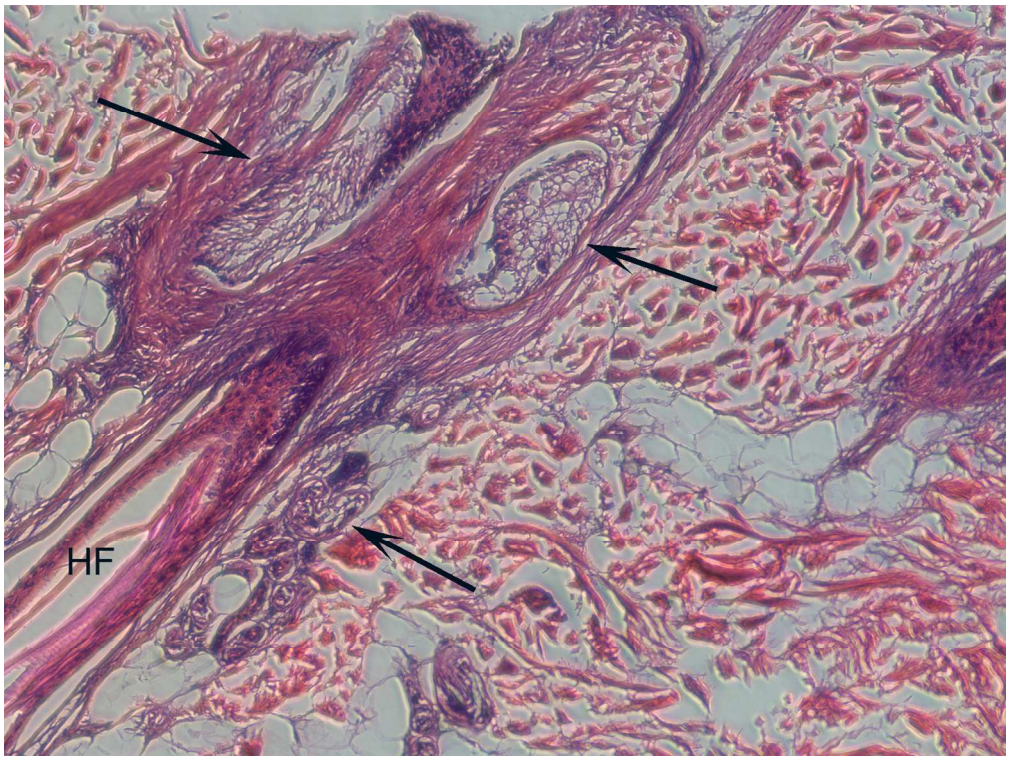
H&E slides at 10x. (A) control;
564x423mm (72 x 72 DPI)



(B) 1700 nm laser exposed sites at 100 ms,
564x423mm (72 x 72 DPI)



(C) 125 ms
564x423mm (72 x 72 DPI)



(D) 135 ms. Thicken and hyperchromatic collagen bundles around hair follicles (HF) and loss of structure of sebocytes are consistent with selective photothermolysis of sebaceous glands (arrows). There was not evidence of epidermal or superficial dermal injury.
564x423mm (72 x 72 DPI)