Feasibility of ablative fractional laser-assisted drug delivery with optical coherence tomography

Chih-Hsun Yang,1,2,* Meng-Tsan Tsai,3,4,7 Su-Chin Shen,2,5 Chau Yee Ng,1,2 and Shih-Ming Jung2,6

1 Department of Dermatology, Chang Gung Memorial Hospital, 5 Fusing St., Kwei-Shan, Tao-Yuan, 33302, Taiwan
2 College of Medicine, Chang Gung University, 259, Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan, 33302 Taiwan
3 Department of Electrical Engineering, School of Electrical and Computer Engineering, College of Engineering, Chang Gung University, 259, Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan, 33302 Taiwan
4 Graduate Institute of Electro-Optical Engineering, School of Electrical and Computer Engineering, College of Engineering, Chang Gung University, 259, Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan, 33302 Taiwan
5 Department of Pathology, Chang Gung Memorial Hospital, 5 Fusing St., Kwei-Shan, Tao-Yuan, 33302 Taiwan
6 Department of Ophthalmology, Chang Gung Memorial Hospital, 5 Fusing St., Kwei-Shan, Tao-Yuan, 33302 Taiwan

Abstract: Fractional resurfacing creates hundreds of microscopic wounds in the skin without injuring surrounding tissue. This technique allows rapid wound healing owing to small injury regions, and has been proven as an effective method for repairing photodamaged skin. Recently, ablative fractional laser (AFL) treatment has been demonstrated to facilitate topical drug delivery into skin. However, induced fractional photothermolysis depends on several parameters, such as incident angle, exposure energy, and spot size of the fractional laser. In this study, we used fractional CO2 laser to induce microscopic ablation array on the nail for facilitating drug delivery through the nail. To ensure proper energy delivery without damaging tissue structures beneath the nail plate, optical coherence tomography (OCT) was implemented for quantitative evaluation of induced microscopic ablation zone (MAZ). Moreover, to further study the feasibility of drug delivery, normal saline was dripped on the exposure area of fingernail and the speckle variance in OCT signal was used to observe water diffusion through the ablative channels into the nail plate. In conclusion, this study establishes OCT as an effective tool for the investigation of fractional photothermolysis and water/drug delivery through microscopic ablation channels after nail fractional laser treatment.

©2014 Optical Society of America

OCIS codes: (110.4500) Optical coherence tomography; (290.1350) Backscattering; (170.2655) Functional monitoring and imaging; (170.1870) Dermatology.

References and links

1. M. Kinoshita, N. McDannold, F. A. Jolesz, and K. Hynynen, “Noninvasive localized delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood-brain barrier disruption,” Proc. Natl. Acad. Sci. U.S.A. 103(31), 11719–11723 (2006).
2. H. L. Liu, M. Y. Hua, H. W. Yang, C. Y. Huang, P. C. Chu, J. S. Wu, I. C. Tseng, J. J. Wang, T. C. Yen, P. Y. Chen, and K. C. Wei, “Magnetic resonance monitoring of focused ultrasound/magnetic nanoparticle targeting delivery of therapeutic agents to the brain,” Proc. Natl. Acad. Sci. U.S.A. 107(34), 15205–15210 (2010).
3. J. S. Woo, I. K. Rhee, and H. D. Park, “Differential damage in bacterial cells by microwave radiation on the basis of cell wall structure,” Appl. Environ. Microbiol. 66(5), 2243–2247 (2000).
4. M. A. Vandelli, M. Romagnoli, A. Monti, M. Gozzi, P. Guerra, F. Rivasi, and F. Forni, “Microwave-treated gelatin microspheres as drug delivery system,” J. Control. Release 96(1), 67–84 (2004).
5. K. van der Maaden, W. Jiskoot, and J. Bouwstra, “Microneedle technologies for (trans)dermal drug and vaccine delivery,” J. Control. Release 161(2), 645–655 (2012).
6. T. Ratanapak, J. Birchall, K. Young, M. Ishii, I. Mieginski, T. Rades, and S. Hook, “Transcutaneous immunization using microneedles and cubosomes: Mechanistic investigations using Optical Coherence Tomography and Two-Photon Microscopy,” J. Control. Release 172(3), 894–903 (2013).
7. T. S. Alster and S. Garg, “Treatment of facial rhytides with a high-energy pulsed carbon dioxide laser,” Plast. Reconstr. Surg. 98(5), 791–794 (1996).
8. C. B. Zachary, “Modulating the Er:YAG laser,” Lasers Surg. Med. 26(2), 223–226 (2000).
9. E. V. Ross, F. P. Sajben, J. Hsia, D. Barnette, C. H. Miller, and J. R. McKinlay, “Nonablative skin remodeling: Selective dermal heating with a mid-infrared laser and contact cooling combination,” Lasers Surg. Med. 26(2), 186–195 (2000).

10. D. Manstein, G. S. Herron, R. K. Sink, H. Tanner, and R. R. Anderson, “Fractional photothermolysis: A new concept for cutaneous remodeling using microscopic patterns of thermal injury,” Lasers Surg. Med. 34(5), 426–438 (2004).
11. H. J. Laubach, Z. Tannous, R. R. Anderson, and D. Manstein, “Skin responses to fractional photothermolysis,” Lasers Surg. Med. 38(2), 142–149 (2006).
12. P. F. Hsiao, Y. C. Lin, C. C. Huang, and Y. H. Wu, “Efficacy and safety of a single treatment using a 10,600-nm carbon dioxide fractional laser for mild-to-moderate atrophic acne scars in Asian skin,” Dermatol. Sin. 31(2), 59–63 (2013).
13. W. R. Lee, S. C. Shen, S. A. Al-Suwayeh, H. H. Yang, C. Y. Yuan, and J. Y. Fang, “Laser-assisted topical drug delivery by using a low-fluence fractional laser: Imiquimod and macromolecules,” J. Control. Release 153(3), 240–248 (2011).

14. M. Charkhchian, A. Beheshti, A. A. Zangivand, and A. Sedighi, “Nail disorder among patients on maintenance hemodialysis,” Dermatol. Sin. 31(1), 7–10 (2013).
15. E. H. Lim, H. R. Kim, Y. O. Park, Y. Lee, Y. J. Seo, C. D. Kim, J. H. Lee, and M. Im, “Toenail onychomycosis treated with a fractional carbon-dioxide laser and topical antifungal cream,” J. Am. Acad. Dermatol. 70(5), 918–923 (2014).
16. D. Huang, E. A. Swanson, C. P. Lin, J. S. Schuman, W. Chang, M. R. Hee, T. Flotte, K. Gregory, C. A. Puliafito, and J. G. Fujimoto, “Optical Coherence Tomography,” Science 254(5035), 1178–1181 (1991).
17. A. Ahmad, N. D. Shemonski, S. G. Adie, H. S. Kim, W. M. W. Hwa, P. S. Carney, and S. A. Boppart, “Real-time in vivo measurement of cornea and anterior segment by office-based polarization-sensitive optical coherence tomography,” Nat. Photonics 7(6), 444–448 (2013).
18. M. A. Choma, M. V. Sarunic, C. H. Yang, and J. A. Izatt, “Sensitivity advantage of swept source and Fourier domain optical coherence tomography,” Opt. Express 11(18), 2183–2189 (2003).
19. S. H. Yun, G. J. Tearney, B. E. Bouma, B. H. Park, and J. F. de Boer, “High-speed spectral-domain optical coherence tomography at 1.3 µm wavelength,” Opt. Express 11(26), 3598–3604 (2003).
20. H. Choi, H. Hiro-Oka, K. Shimizu, and K. Ohbayashi, “Spectral domain optical coherence tomography of multi-MHz A-scan rates at 1310 nm range and real-time 4D-display up to 41 volumes/second,” Biomed. Opt. Express 3(12), 3067–3086 (2012).
21. S. H. Yun, G. J. Tearney, J. F. de Boer, N. Ifitinia, and B. E. Bouma, “High-speed optical frequency-domain imaging,” Opt. Express 11(22), 2953–2963 (2003).
22. I. Grulowski, J. J. Liu, B. Potsaid, V. Jayaraman, J. Jiang, J. G. Fujimoto, and A. E. Cable, “High-precision, high-accuracy ultralong-range swept-source optical coherence tomography using vertical cavity surface emitting laser light source,” Opt. Lett. 38(5), 673–675 (2013).
23. W. Wieser, W. Draxinger, T. Klein, S. Karpf, T. Pfeiffer, and R. Huber, “High definition live 3D-OCT in vivo: design and evaluation of a 4D OCT engine with 1 GVoxel/s,” Biomed. Opt. Express 5(9), 2963–2977 (2014).
24. S. Makita, T. Fabritius, and Y. Yasuno, “Full-range, high-speed, high-resolution 1-µm spectral-domain optical coherence tomography using BM-scan for volumetric imaging of the human posterior eye,” Opt. Express 16(12), 8406–8420 (2008).
25. Y. Lim, M. Yamanari, S. Fukuda, Y. Kaji, T. Kiuchi, M. Miura, T. Oshika, and Y. Yasuno, “Birefringence measurement of cornea and anterior segment by office-based polarization-sensitive optical coherence tomography,” Biomed. Opt. Express 2(8), 2392–2402 (2011).
26. A. Unterhuber, B. Povazay, A. Müller, O. B. Jensen, M. Dueil, T. Le, P. M. Petersen, C. Velez, M. Esmaeelpour, P. E. Andersen, and W. Drexler, “Simultaneous dual wavelength eye-tracked ultrahigh resolution retinal and choroidal optical coherence tomography,” Opt. Lett. 38(21), 4312–4315 (2013).
27. A. Bradu and A. G. Podoleanu, “Imaging the eye fundus with real-time en-face spectral domain optical coherence tomography,” Biomed. Opt. Express 5(4), 1233–1249 (2014).
28. A. Alex, J. Weingast, M. Weinigel, M. Kellner-Höfer, R. Nemecek, M. Binder, H. Pehamberger, K. König, and W. Drexler, “Three-dimensional multiphoton/optical coherence tomography for diagnostic applications in dermatology,” J. Biophotonics 6(4), 352–362 (2013).
29. E. Sattler, R. Kästle, and J. Welzel, “Optical coherence tomography in dermatology,” J. Biomed. Opt. 18(6), 061213 (2013).
1. Introduction

Recently, a lot of studies have been focusing on the technical improvement in drug delivery such as focused ultrasound [1,2], microwave [3,4], and microneedle patch [5,6]. With exposure of focused ultrasound, the vascular permeability can be enhanced, making drug delivery from the vessel to surrounding tissue easier. Currently, focused ultrasound is applied to brain blood barrier opening. Low-temperature microwave opens up pores in bacteria cells, inducing significant improvement in drug delivery. Moreover, microneedle patch bypasses stratum corneum barrier by producing microscopic holes in the epidermis, facilitating drug diffusion into dermal microcirculation. Mostly, focused ultrasound with microbubbles is implemented for increasing the vascular permeability to facilitate drug delivery from the vessels to surrounding tissue. For transdermal drug delivery, microwave technique is still not mature, according to the previous studies. In addition, limited to the size and material of microneedle patch, the efficiency of microneedle-assisted transdermal drug delivery is easily influenced. Therefore, the abovementioned methods can only be applied in limited situations.

In the past, laser skin resurfacing was performed by scanning the whole skin surface with laser beams [7–9]. This treatment frequently leads to delayed skin recovery as the entire
epidermis was removed. Fractional photothermolysis was developed to replace traditional full-surface skin resurfacing. Treatment with the Fractional CO\textsubscript{2} laser (10600 nm) laser creates microscopic ablation zones (MAZs) that extend into dermis and are surrounded by intact viable tissue, allowing rapid healing [10–12]. Beside aesthetic treatment, fractional laser ablation has been shown to efficiently disrupt stratum corneum and facilitate transcutaneous drug and vaccine delivery. Arrays of spatially separated microscopic wound generated by fractional laser can provide free paths for drug delivery into the skin [13]. Therefore, topical drug is the most preferred therapy option due to rare adverse reactions and better patient compliance. Among the most common disorders of nail is onychomycosis, a fungal infection of the nail plate or bed [14]. However, nail drug delivery constitutes a major challenge as the nail is composed of hard, densely keratinized nail plate that functions as a barrier, impairing drug penetration to the nail bed. A variety of physical methods or chemical enhancers have been tested to increase drug permeation into the intact nail plate. Currently, ablative fractional laser that generates many tiny micro-channels in the nail proved to be an effective method for nail drug delivery [15].

Optical coherence tomography (OCT) has been widely used as a diagnostic tool in clinical trials and animal studies, due to the natures of noninvasive scanning, high-speed imaging, high spatial resolution and no extraneous agent needed [16,17]. With the development of Fourier-domain OCT, including spectral-domain OCT (SD-OCT) and swept-source OCT (SS-OCT), can provide a high imaging sensitivity in biological tissue [18–23]. Currently, OCT has become a clinical approach for various disease diagnoses, such as ophthalmology [24–27], dermatology [28–36], cardiology [37,38], and gastrointestinal tract [39,40]. For dermatology applications, some groups have demonstrated that OCT can be used for diagnoses of skin diseases, skin cancer [28,29], sun damage [30], and burn depth [31]. With polarization-sensitive OCT, the human burn scars can be quantitatively analyzed, and human skin at different ages can be differentiated [32–35]. Moreover, OCT-based angiography has been used for identification of skin diseases [36]. In addition, several approaches have been proposed to monitor or evaluate the laser tissue ablation, including histology [41], confocal microscopy [42], harmonic microscopy [43] and OCT [44]. Although histology is a golden standard to obtain tissue morphology, it is an invasive and time-consuming method. With confocal/harmonic microscopy, the imaging depth is limited to a depth range of less than 300 μm. In addition, using fractional laser to ablate the tissue can induce an ablation depth of more than 1 mm, which is difficult to probe the deeper structures of MAZs with confocal/harmonic microscopy. In our previous study, OCT was used as a monitoring tool for investigating the photothermolysis in human skin induced by non-ablative and ablative fractional lasers. Furthermore, OCT was implemented for \textit{in vivo} investigation of wound healing after non-ablative and ablative fractional laser exposures.

In the previous results, OCT offers noninvasive, real-time visualization of the different layers of the skin in almost histopathological resolution \textit{in vivo}. OCT imaging enables to confirm the depth of penetration and density of these microscopic wound spots. In this study, we propose to use OCT for noninvasive monitoring the treatment outcome of AFL and evaluate the feasibility of water diffusion through the induced ablative channels. With OCT scanning, the penetration depth of laser spot can be quantitative evaluated, enabling to optimize the feasible laser energy parameters in treating nail diseases. The OCT results were also compared with the results of histology and dermoscopic examination. In addition, to understand the feasibility of fractional laser-assisted drug delivery, speckle variance in OCT signal is used to observe and to estimate water diffusion process through the ablative channels into the nail plate. Finally, the water diffusion process is also discussed. The utilization of OCT in the evaluation of nail fractional laser treatment is a promising technique for future medical applications in the treatment of nail diseases such as nail fungus infection.
2. System setup and experimental methods

2.1 OCT system setup

Fig. 1. Schematic diagram of OCT system. FC: fiber coupler; CIR: circulator; G: two-axis galvanometer; SMF: single-mode fiber; DC: dispersion compensator; M: mirror, and OB: objective lens. The physical area of OCT imaging was set to be approximately 2.5 × 2.5 mm².

In this study, we demonstrated an SS-OCT system with a scanning probe for skin imaging, as shown in Fig. 1. The central wavelength of the wavelength-sweeping laser (HSL-20, Santec Corp., Japan) is located at 1310 nm with a scanning range of 105 nm, which corresponds to a theoretical resolution in the longitudinal direction of ~7 μm. The laser source can provide a scanning rate of 100 kHz and an output power of 20 mW, respectively. Then, the output light was connected to a Mach-Zehnder interferometer, composed of two circulators and two couplers. For skin scanning, a handheld probe with three-dimensional imaging ability was fabricated, which was composed of a collimator, a reflective prism, a two-axis galvanometer and a 10 × objective lens (LSM02, Thorlabs Inc., New Jersey, US). In our system, the transverse resolution is approximately 7 μm. In order to compensate the dispersion induced by the objective lens used in the scanning probe, a dispersion compensator was inserted in the reference arm. Finally, the interference signal combined from the sample and reference arms was detected by a balanced detector. To resample the interference spectrum, a k-clock signal generated from the light source was utilized as an external clock and received by a digitizer (ATS9350, Alazar Technologies Inc., Canada). To scan human skin, the physical area of OCT imaging was set to be approximately 2.5 × 2.5 × 3 mm³, consisting of 1000 × 500 × 600 voxels. Based on an A-scan rate of 100 kHz, the frame rate of our OCT system can achieve 100 frames/s, in which each frame is composed of 1000 A-scans.

2.2 Patients

This study was conducted in the outpatient clinic of the Dermatology Department of the Chang Gung Memorial Hospital in Taipei, Taiwan. The study was approved by the Chang
Gung Medical Foundation Institutional Review Board (No. 101-2921A3). After written informed consent, participants received a standardized application of fractional ablative laser treatment using the CO$_2$ laser (UltraPulse Encore Active FX™; Lumenis, Santa Clara, Calif. US) with a central wavelength of 10600 nm. In the Deep Fx mode, a precise matrix pattern of micropore can be produced using a single pass of laser. The setting of laser parameters was the following: shape of the scanned field: square-shaped, size of scanned field: 6 mm × 6 mm, pulse duration: 3.5 ms, fraction coverage: 10%. Each laser treatment covered approximately 3.6 cm$^2$, with pulse energy settings at 20, 30, 40, and 50 mJ. All the procedures were done without topical anesthesia. There were no discomforts during the laser treatment. The procedure can be done within 5 minutes for 10 fingernails.

3. Results

3.1 Dermoscope, histologic, and OCT results

![Fig. 2. (a) Top-view and (b) side-view images of a finger nail treated with a pulse energy of 30 mJ. (c) Top-view and (d) side-view images of a finger nail treated with a pulse energy of 40 mJ. The pictures show clearly demarcated and cylindrical MAZs that extended into the nail plate.](image)

Firstly, to study fractional photothermolysis induced by an ablative fractional laser (AFL), four finger nails of the same female volunteer were exposed by an AFL with various exposure energies of 20, 30, 40, and 50 mJ. After AFL exposures, dermoscope images (Heine Delta 20, Herrsching, Germany) were obtained using digital camera (Nikon D5300, Tokyo, Japan) with a 10 × magnification on the AFL-treated nails prior to in vivo investigation of AFL effect with OCT [45]. Figure 2 shows the dermoscope images of the nails exposed to AFLs. A square matrix pattern of micropore on the nail plate can be visualized with naked eyes. Under dermoscope examination, each of these figures shows clearly demarcated and punch-out microhole on the nail surface. Dermoscopy of the free edge (distal edge) demonstrated arrays of cylindrical MAZs that extended into the nail plate, these MAZs were due to tissue volatilization induced by the AFL.

After dermoscope examination, the treated nails were sequentially scanned with the OCT system to obtain 3D structural images. Figures 3(a)-3(d) show the 2D OCT results of the same nails. The white arrows indicate the MAZs induced by AFL, due to the nail volatilization resulting in weakly backscattered signal. The red arrows in Figs. 3(a)-3(d) represent the maximum penetration depths induced by AFL, which can be estimated from OCT signals to be given as 248 μm, 290 μm, 320 μm, and 368 μm, respectively. From the results, one can see that the penetration depth is proportionate to the exposure energy. Furthermore, to further understand the induced ablative area of each spot, en-face images were obtained from 3D OCT images. Figures 3(e)-3(h) show the en-face images at the nail surface, which were obtained from the same nails of Figs. 3(a)-3(d), exposed by various
exposure energies of 20, 30, 40, and 50 mJ. The results showed that the ablative area increases with increasing exposure energy.

![Fig. 3. (a)-(d) 2D OCT images of four nails after AFL exposures with energies of 20, 30, 40, and 50 mJ. (e)-(h) En-face images at the nail surface, which were obtained from 3D OCT images of (a)-(d). (i)-(l) paraffin embedded H&E histology images of the same nails in (a)-(d). The white arrows indicate the induced MAZ structures.](image)

After OCT scanning, all treated nails were clipped and underwent histologic examination. After softening the nail specimen with 7.3% trichloroacetic acid solution for 1 day, the samples were embedded in paraffin then sliced into 3 μm thick sections and stained with hematoxylin and eosin (H&E). Since small variations in the angle of sectioning with the microtome could mean significant differences in the apparent depth of the MAZs, we obtained the maximum depth of lesions that were representative of each pulse energy parameter for the specimen. Histologic evaluation of the laser treated nail plate demonstrates the formation of microscopic hole inside the nail plate. Figures 3(i)–3(l) represent paraffin embedded H&E histology images of the nails with AFL exposures with energies of 20, 30, 40, and 50 mJ, respectively. The white arrows in Figs. 3(i)-3(l) indicated the structures of induced MAZs. The histological results showed that the penetration depth of laser spot increases with increasing exposure energy, which is consistent with the OCT results. The depth of penetration per histology was 266 μm with 20 mJ energy exposure, 303 μm with 30 mJ, 332 μm with 40 mJ, and 359 μm with 50 mJ.

3.2 Quantitative evaluation of MAZs

Subsequently, the mean depths of MAZs induced by various exposure energies in Fig. 3 were estimated from OCT and histologic results, as shown in Fig. 4(a). The depth of MAZ deepens with increasing exposure energy and in proportion with the laser energy emitted. Figure 4(b) shows the statistical result of MAZ diameter. In Fig. 4(b), the mean diameter of induced MAZs also increases with the increasing exposure energy, due to that the stronger photothermolysis induced by the higher exposure energy caused more surrounding tissue ablated. In general, no significant difference was found between the depths of the MAZ obtained by OCT versus H&E (P<0.05), validating OCT as an acceptable methodology for
fractional lesion depth evaluation. Here, volatilization induced by AFL causes the deposition in the deeper portion of MAZ, resulting in stronger backscattered signal from the deeper portion of MAZ, as shown in Figs. 3(a)-3(d). In contrast, the deposition can be removed during histologic process, as mentioned in section 3.1. In addition, the induced photothermolysis depends on the exposure parameters, such as the exposure energy, the incident angle, the spot density, the optical property of tissue, etc. Moreover, the nail is a curved structure, which makes the same exposure condition at the different nail locations difficult to be maintained. According to the abovementioned factors, OCT results showed the larger standard deviations in the penetration depth and the diameter of MAZs.

![Fig. 4. (a) Plot of mean MAZ depths, and (b) plot of mean MAZ diameter following CO₂ fractional laser treatment of finger nails at various pulse energies of 20, 30, 40, and 50 mJ.](image)

### 3.3 Observation of diffusion process

To investigate the feasibility of fractional laser-assisted drug delivery, normal saline was dropped on the exposed area of a finger nail (36-year-old male), which was exposed by an energy level of 35 mJ. With AFL exposure, an exposure area of 6 mm × 6 mm was produced and then, the exposure area was in vivo scanned with the OCT system. In order to observe the diffusion process through the induced MAZs, the same location was continuously scanned with the OCT system before and after dropping normal saline. Here, the OCT scanning range was set to be 2 mm along the transverse direction, consisting of 1000 A-scans/frame. Based on the A-scan rate of 100 kHz in our OCT system, the OCT system can achieve a frame rate of 100 frames/s. However, it is difficult to observe the saline water diffusion process from original 2D OCT images. Therefore, in this study, we proposed to evaluate the speckle variance of OCT signal, resulting from the water diffusion.

In previous studies, speckle-variance OCT (SV-OCT) is widely used for angiography, due to the variation in the backscattered intensity from the blood cells [46–49]. Compared with other OCT-based angiography techniques such as phase-resolved OCT and Doppler variance OCT, SV-OCT is not sensitive to the blood velocity or the angle between the optical beam and the blood vessel. Therefore, SV-OCT can be used for estimating the location of moving particle according to the speckle variance in OCT signal. Here, the speckle variance \( (SV_{ijk}) \) can be estimated as

\[
SV_{ijk} = \frac{1}{N} \sum_{i=1}^{N} (I_{ijk} - \frac{1}{N} \sum_{i=1}^{N} I_{ijk})^2
\]

where \( i \) and \( j \) represent the transverse and depth indices of each frame [46]. \( N \) is the total number of B-scan frames used for speckle-variance estimation. Normally, the moving particles in the biological tissue results in speckle variance in OCT signals. However, in our experiment, because there is no vessel structure in the nail structure, the variation in OCT speckle signal results from the extraneously moving particles or system noise. Therefore, to
remove the speckle noise, the successive OCT images were recorded to evaluate the noise level of speckle variance and a threshold value of the noise level of speckle variance can be determined, which also reject the vascular signal below the nail structure. Subsequently, to evaluate the time-resolved variation in OCT speckle signal, a 2D OCT image of the treated nail before dropping the normal saline was recorded as the reference. Then, OCT images at the same location of the treated nail were successively recorded after dropping normal saline on the exposed area. Then, each OCT image was used to estimate the speckle variance with the reference image (obtained before dropping normal saline on the treated nail), according to Eq. (1). Thus, in our experiments, $N$ equals 2 (the reference OCT image and the OCT image obtained at arbitrary time point). Finally, the estimated speckle variance image was merged with the original OCT image to become an SV-OCT image, making easily map the location of the presence of speckle variance.

Fig. 5. Time-resolved SV-OCT images of the nail without fractional laser exposure obtained (a) before dropping the normal saline, and after dropping the normal saline at (b) 60 s, (c) 120 s, (d) 180 s, (e) 240 s, and (f) 300 s. The white arrow indicates the nail plate (the dark stripe), and the yellow arrow represents the image artifact in our system.

Firstly, a finger of the volunteer without fractional laser exposure was fixed on a specially designed mount to avoid unconscious motion and the same location of nail was in vivo scanned with the OCT system. Then, successive B-scans were recorded before and after dropping the normal saline on the nail. Sequentially, the abovementioned procedure was performed to acquire time-resolved SV-OCT images. In the proposed method for the evaluation of speckle variance signal, an OCT image of the nail before dropping the normal saline was recorded as the reference, and each B-scan was used to estimate the speckle variance with the reference image. Figure 5 shows the time-resolved SV-OCT results of the nail without fractional laser exposure. Here, the white arrow indicates the nail plate and the yellow arrow represents the artifact in our OCT system. From the results, no significant increase in speckle variance can be found. At 150 s, the speckle variance slightly appeared on the nail surface. The results showed that the normal saline is difficult to diffuse into the nail from the intact nail surface.

Then, another fingernail of the same volunteer after AFL exposure was in vivo scanned with the OCT system. Figure 6 shows the representative SV-OCT images obtained at different time points. The upper figure in Fig. 6 shows the time scale of Figs. 6(a)-6(o). Figure 6(a) was an SV-OCT image obtained before dropping the normal saline on the treated nail by estimating the speckle variance of two adjacent OCT images (One of two images was used as the reference image). From Fig. 6(a), no significant speckle variance can be found. In
addition, Figs. 6(b)-6(o) represent the representative SV-OCT images obtained at different time points of 0.4, 0.8, 2, 4, 8, 16, 34, 38, 42, 60, 80, 100, 120, and 140 s. The successive diffusion process for a period of 140 s can be found in Media 1. In Fig. 6(a), the red arrow indicates the nail plate and the area above the nail plate is the nail structure with induced MAZs. In addition, the white arrow in Fig. 6(a) shows the water drop. With the time-resolved SV-OCT images, the speckle variance signal started to occur at 0.8 s and gradually increased with time. In Fig. 6(f), the stronger speckle variance signal can be observed in the left portion of figure. Subsequently, the speckle variance signal emerged in the right portion of OCT image. In Fig. 6(m), the water drop became smaller, indicated by the white arrow, which is probably due to the water evaporation or diffusion into the nail structure. In contrast, no significant speckle variance can be observed before dropping normal saline on the treated nail, as shown in Fig. 6(a). Therefore, the occurrence of speckle variance signal was due to the water diffusion, proving that MAZs can effectively improve water diffusion or drug diffusion.

4. Discussion

OCT is a well-used microscopic tool that provides valuable morphological and functional information of tissues, enabling to reconstruct a depth-resolved structural image of biological tissue in a depth range of 2-3 mm. In addition to structure reconstruction, functional OCT techniques are also developed including angiography, birefringence, and elasticity. Because OCT can probe the deeper structure when compared with microscopy techniques, such as confocal microscopy, multiphoton microscopy and harmonics generation microscopy, OCT has been widely used for the studies on dermatology, enabling to analyze the different layer structures of skin. However, most studies in dermatology with OCT were only focusing on
the diagnosis of skin diseases. In this study, we used OCT as a monitoring tool for quantitative evaluation of fractional photothermolysis reaction on the nail. The thicknesses and optical property of nails varies, resulting in distinct induced fractional photothermolysis in different subjects. Thus, a monitoring tool for real-time evaluation is warranted.

The success of transdermal drug delivery has been severely limited by the inability of most drugs to penetrate the skin barrier. A variety of methods (e.g., microneedles, tape stripping, chemical peeling, and ultrasound) have been tested in the past decade and these modalities act by disrupting epidermis to facilitate trans-epidermal drug delivery. Delivering drug into the nail is even more challenging because of hard, densely keratinized nail plate. Laser-assisted drug delivery is an evolving technology with potentially broad clinical applications. Fractional CO2 Laser is a device that emits a 10600-nm laser able to penetrate deeply into the skin by ablating narrow, vertical channels to a selected depth. In this study, we demonstrated that fractional CO2 laser enhances drug penetration by disrupting nail plate and the depth of penetration can be monitored by OCT. Because onychomycosis is a fungal infection of the fingernails or toenails, it causes discoloration, thickening, and separation from the nail bed. Moreover, onychomycosis occurs in 10% of the general population but is more common in older adults. Oral medications are the most effective treatment option with cure rates from 50% to 80%. However, oral medications are limited by the risk of serious adverse side events such as hepatotoxicity and potential drug interactions. Topical antifungals are often ineffective in the treatment of onychomycosis due to their inability to penetrate the nail plate. In this study, we have demonstrated here that fractional CO2 laser with energy setting at 50 mJ can creates vertical channels depths exceeded 0.35 mm into the finger nail plate that might assist the delivery of topically applied drugs into nail.

5. Conclusion

In conclusion, this study illustrates the correlation of nail morphology after fractional laser therapy through the utilization of OCT. With OCT, the photothermolysis of MAZs can be quantitatively evaluated, enabling real-time monitoring of the exposure outcome and determine the optimal laser treatment energy settings. Moreover, we also show the preliminary result to observe liquid (normal saline) diffusion process through the induced MAZs with the use of SV-OCT, proving the feasibility of fractional laser-assisted drug delivery. The liquid diffusion process can be further applied to drug diffusion studies in the future.

Acknowledgment

This research was supported by the National Science Council (NSC), and Chang Gung Memorial Hospital, Taiwan, the Republic of China, under the MOST 103-2221-E-182A-001-, MOST 103-2221-E-182 –039-, and CMRPD2B0032 grants.