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Optical Coherence Tomography for *In Vitro* Monitoring of Wound Healing After Laser Irradiation

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***Abstract*—We demonstrate a novel application of optical coherence tomography (OCT) to monitor post-laser irradiation collagen injury in model skin. An artificial skin model (RAFT), which closely approximates human skin, was irradiated with a Perovskite laser ($\lambda = 1341$ nm), which is under investigation for potential use as a nonablative laser skin rejuvenation device (NALSR). OCT was used to determine the extent of laser injury immediately post irradiation and, subsequently, to monitor tissue recovery over a seven-day period. OCT images clearly delineated areas of post-irradiation collagen injury and allowed noninvasive monitoring of the wound healing process. Histology was used for comparison and correlated well with OCT images. OCT offers advantages over standard histology as it is noninvasive and allows serial monitoring at the same site over time. Our results indicate that OCT has potential as a method for characterization of collagen injury post-laser irradiation and may be a useful tool for determination of optimal parameters for NALSR using different devices under investigation for this indication.**

***Index Terms*—Artificial skin model (RAFT), histology, nonablative laser skin rejuvenation (NALSR), optical coherence tomography.**

I. Introduction

DIRECT visualization of tissue structure offers a unique opportunity for evaluation of tissue effects after laser irradiation. Optical coherence tomography (OCT) is a potential modality [1]–[3] to monitor the laser-induced wound healing response after irradiation. During the last decade, nonablative laser skin rejuvenation (NALSR) has become a popular procedure for treatment of fine facial wrinkles and acne scars [4]–[7]. Laser irradiation is used to heat the dermis, resulting in collagen coagulation and stimulation of a wound healing process, which is intended to induce collagen remodeling and neo-collagen formation. A variety of lasers and light source have been evaluated for their potential as NALSR devices [4], [7]. Variable treatment success has been achieved, but to date, an optimal method for NALSR has not been developed [4]. Many treatment parameters must be considered during development and testing of new NALSR devices including wavelength, pulse duration, focal characteristics of the light, and number of pulses. To develop effective devices for this clinical indication, it is imperative to noninvasively and dynamically evaluate the tissue wound healing response after laser irradiation. Currently, biopsies and histologic evaluation are used to study the effects of laser irradiation. However, biopsy has difficulties including potential structural changes to the tissue during the excision and the inability to serially image the same site as a function of time [8], [9].

OCT is a relatively new imaging modality based on detection of backscattered light from biological samples. OCT is analogous to ultrasound except that imaging is performed with light instead of acoustic waves. OCT utilizes a Michelson interferometer with a low coherence source to measure light reflected from turbid structures and is capable of performing high-resolution ($\sim 10\ \mu$m), cross-sectional imaging. OCT enables real-time, *in situ* visualization of tissue microstructure without the need to excise and process the specimen as required for conventional biopsy and histopathology. Consequently, OCT is a powerful method to image noninvasively biological tissue over time in response to laser irradiation.

In the present study, we have used OCT to monitor in an organotypic RAFT model of the skin. The RAFT tissue culture model of human skin [10], [11] was irradiated with a Perovskite laser ($\lambda = 1341$ nm) and the wound healing response followed over a seven-day period using OCT and conventional histopathology.

II. Materials and Methods

A. Model Preparation

An organotypic (RAFT) model of the skin was used to study the effect of laser irradiation on wound healing response. The RAFT skin model was composed of human dermal fibroblast cells in a collagen type I gel with surface epithelia of human

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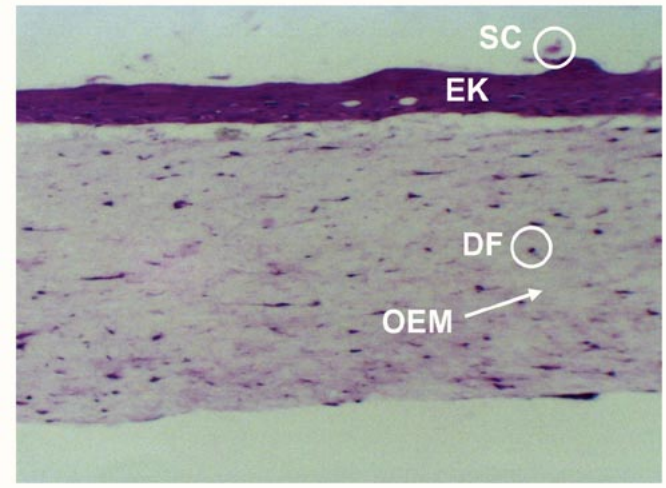

Fig. 1. Histology of model skin. Stratum corneum (SC). Epidermal keratinocytes (EK). Dermal fibroblasts (DF). Organized extracellular matrix (OEM).

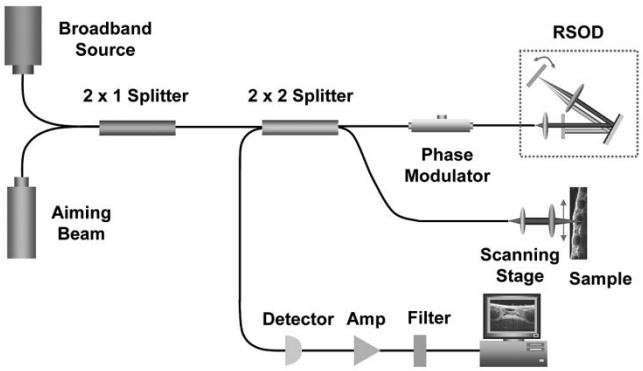

Fig. 2. Schematic of OCT imaging system: rapid-scanning optical delay (RSOD).

epithelial keratinocytes. We have cultured keratinocyte, fibroblast, and microvascular endothelial cells from neonatal foreskin tissue and used them to reconstitute a human skin model (fibroblast-containing collagen gel simulating dermis with stratified multilayered keratinocytes simulating epidermis).

Cell Cultures: Normal human epidermal keratinocytes from neonatal skin (BioWhittaker, Inc., Walkersville, MD) were cultured in KGM-2 medium (Invitrogen Corp., Carlsbad, CA) at 37 °C in 7.5% CO_2 atmosphere. Normal human dermal fibroblasts were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 20 IU of penicillin per ml, 20 IU of streptomycin per ml, and 0.4 mM l-glutamine.

RAFT Preparation: Reconstitution buffer was made with 2.2 g $NaHCO_3$ and 4.77 g Hepes in 100-ml 0.05-M NaOH and sterilized with a 0.22-μm filter. Seven parts of rat-tail collagen type 1 (Discovery Labware, Inc., Bedford, MA) were mixed with two parts of 5 $\times$ DMEM and one part of buffer and neutralized with 1 M NaOH to pH 7.4. Fibroblasts were suspended in the collagen solution at a density of 4×10^5 cells/ml. For construction of the dermal layer, 1.5 ml of this suspension was allotted to each well of a 24-well plate (Corning, Inc, Corning, NY) and incubated for 24 h at 37 °C in a 7.5% CO_2 atmosphere. For construction of the epidermal layer, keratinocytes were suspended with KGM-2 medium at a density of 6×10^5 cells/ml and seeded in 0.5-ml aliquots to each dermal layer of a 24-well plate. The following day, the models were lifted to the air-liquid interface using a plastic grid support. The keratinocytes were allowed to stratify and differentiate to form a 50-μm thick epithelium (i.e., 10–12 keratinocyte layers). One week after culture initiation, the skin models were ready for laser irradiation. A representative histological section of the mature model is shown in Fig. 1. Histology of both the epidermis and dermis of RAFT model closely resembles that of human skin.

Laser Irradiation: A 6-mm handpiece was mounted 25 mm above the target surface to irradiate the model skin with one or two pulses from a Perovskite laser ($\lambda = 1341$ nm, Dualis™ by Fotona, Ljubljana, Slovenia). Single-pulse parameters were an energy density of 25 or 35 J/cm^2 and a pulse duration of 20 ms. Double pulses were delivered at a rate of 0.5 Hz.

B. OCT Instrumentation

A schematic of the OCT system is shown in Fig. 2. The time delay of light backscattered from model skin was measured by a fiber based Michelson interferometer. Light was coupled into the interferometer and split into two paths. One beam was directed toward the model skin and the other to a reference mirror. The OCT system used in this study employed a broadband light source that delivered an output power of 10 mW at a central wavelength of 1310 nm with a bandwidth of 70 nm. A visible aiming beam (633 nm) was used to find and locate the exact imaging position on the sample. In the reference arm, a rapid-scanning optical delay line was used that employs a grating to control the phase and group delays separately so that no phase modulation is generated when the group delay was scanned [12], [13]. The phase modulation was generated through an electro-optic phase modulator that produces a carrier frequency. The axial line scanning rate was 400 Hz, and the modulation frequency of the phase modulator was 500 kHz. Reflected beams from the two arms are recombined in the interferometer and detected on a photodetector. The interference signal was observed only when the optical path length difference between sample and reference arms was less than the coherence length of the source. The detected optical interference fringe intensity signals were bandpass filtered at the carrier frequency. Resultant signals were then digitized with an analog-digital converter and transferred to a computer where the structural image was generated. The lateral and axial resolutions of the reconstructed image were 10 and 15 μm, respectively, and determined by the beam waist at the focal point and coherence length of the source.

C. Measurement

For evaluation of the wound-healing response, eight RAFT model specimens were used. Each model was irradiated under identical conditions. One model was imaged daily by OCT over a seven-day period. The imaged area was marked with 100-μm beads so that the exact same position could be relocated every day. Over the seven-day study period, one specimen was harvested each day for histologic analysis. Samples processed for

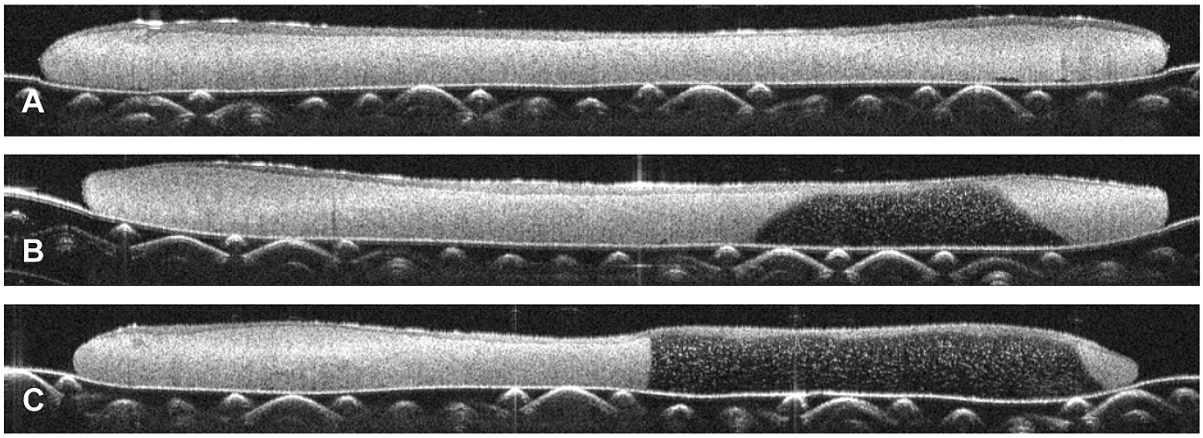

Fig. 3. OCT images of RAFT models (13 × 1.3 mm, 10 μm/pixel). (A) Nonirradiated sample. (B) Single-pulsed control sample. (C) Double pulsed sample.

histopathology were fixed for 24 h in buffered 10% formalin then transferred to a phosphate buffer solution until embedding. Specimens were embedded in paraffin, cut into 6-μm-thick sections and placed on albumin-coated slides for hematoxylin and eosin (HE) staining.

III. Results and Discussions

OCT images of acutely irradiated samples allowed comparison of the effects of single versus double pulsing (Fig. 3). On both laser irradiated samples, a layer of intact epidermis is noted at the top of the model skin. A dark region indicating the laser-irradiated area is clearly observed in both cases. Since OCT measures backscattered light intensity, the dark region, which corresponds to a reduced OCT signal, indicates that the backscattered coefficient is reduced in the laser-irradiated collagen. Collagen in the native state forms super helic bundles and has a large spatial variation of density at the microscopic level. The large spatial variation of density results in a large inhomogeneity of the optical refractive index, which results in a relative large scattering coefficient. Our results suggest that thermal energy deposited by laser irradiation alters collagen structure. The photocoagulated and denatured collagen has a more uniform density distribution, which results in a reduced scattering coefficient. A comparsion of Fig. 3(B) and (C) indicates that double pulse irradiation significantly increase the photocoagulated area.

Fig. 4(A) provides a higher magnification view of the border between injured and intact collagen as imaged by OCT. An H&E stained histology section from the same site is provided for comparison [Fig. 4(B)]. A layer of intact epidermis is noted on both figures. The appearance of the affected collagen in the OCT image results from a black background of altered collagen with condensed fibroblast nuclei noted as scattered white spots. The border between injured and intact collagen is clearly demarcated on the OCT image. This transition is more difficult to ascertain on the histology section because there is a much more gradual change in collagen staining.

Fig. 5 shows a series of OCT images demonstrating the wound healing response to laser irradiation over a seven-day period. Collagen regenerated gradually over time with significant healing achieved by day seven.

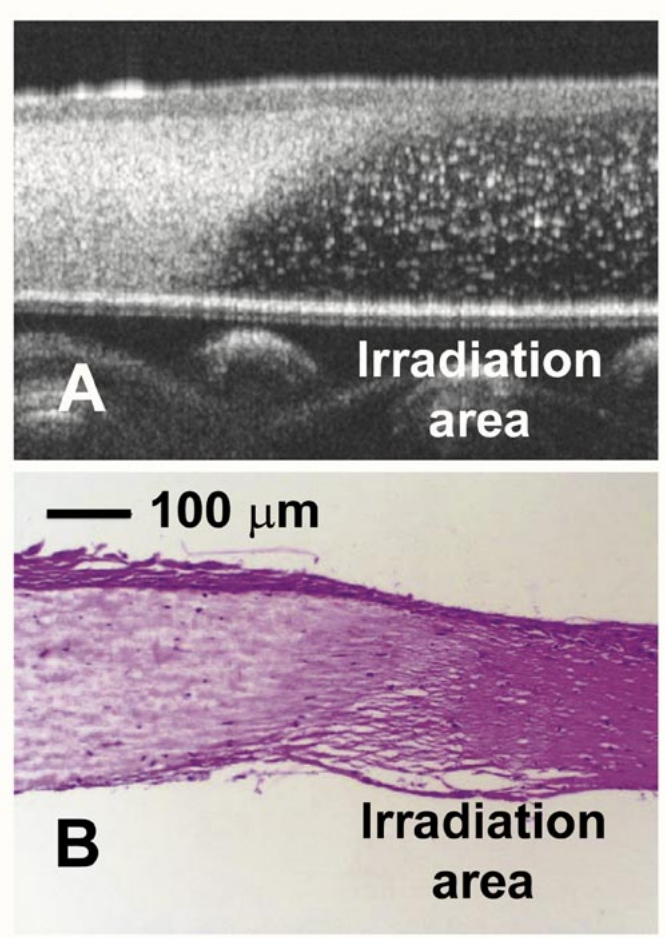

Fig. 4. Comparison of irradiated sample as evaluated by OCT and histology. (A) OCT image (2 × 1.3 mm, 10 μm/pixel). (B) Histology.

In Fig. 6(A), higher magnification views of the OCT images allow closer evaluation of the wound healing response, as seen on days three, five, and seven. Corresponding histology sections are shown for comparison [Fig. 6(B)]. On day three, a relatively large area of injured collagen is noted in the center of the irradiated field. Wound healing is already occurring as evidenced be the presence of fine strands of regenerated collagen and the

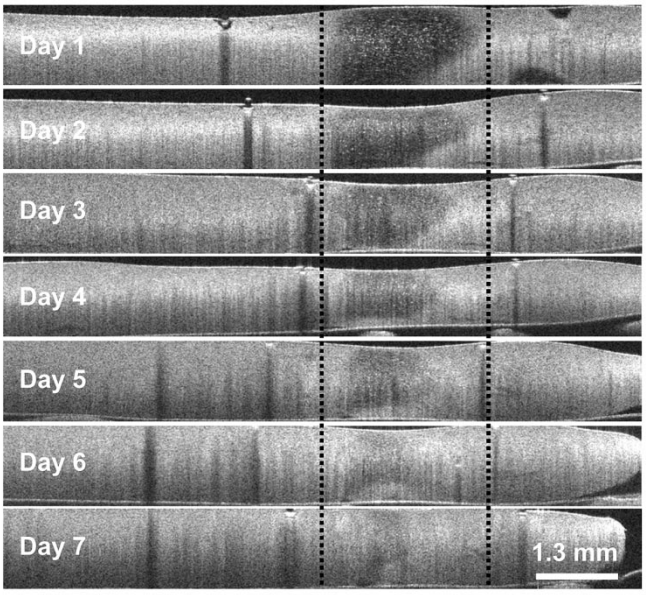

Fig. 5. Wound healing response using OCT of irradiated RAFT over seven days. The dotted lines outline the irradiated region. Bead marks are clearly shown on both sides of the irradiated area. Sample was irradiated by single pulse (25 J/cm^2).

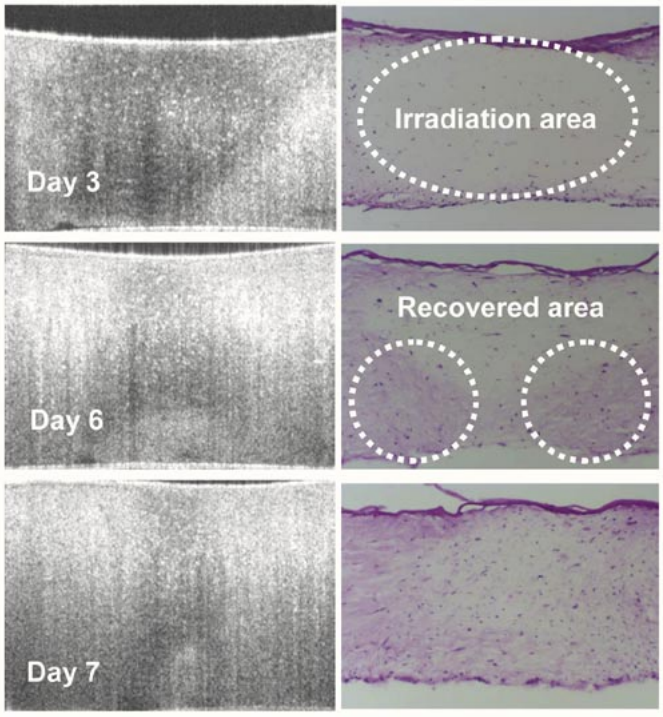

Fig. 6. Comparison between OCT images and histologic sections. OCT image size is 2 $\times$ 1.3 mm with 10 μm/pixel.

migration of fibroblasts into the irradiated area. On day five, more significant collagen regeneration is noted which appears to be initiated from deep and lateral aspects of the specimen, where intact collagen survived laser irradiation. On day seven, further collagen recovery is evident. The above series of images demonstrates the ability of OCT to evaluate collagen photocoagulation post-laser irradiation and to monitor subsequent wound healing and collagen remodeling. OCT imaging of irradiated RAFT model skin offers a method for initial evaluation of potential devices for NALSR. A wide range of laser parameters can be accurately and rapidly evaluated.

It has been suggested that the optimal depth for collagen photocoagulation and induction of collagen remodeling after NALSR is 100–500 μm below the skin surface, where the majority of histologic changes associated with photoaging occur. More superficial injury may be ineffective for rhytides reduction; deeper injury may result in scarring. Once optimal parameters have been determined to target the desired depth with a new laser device, wrinkle or acne scar reduction studies can be performed on human subjects with relative confidence. We believe that OCT might offer an objective method to evaluate this next stage of the development process as well, but further studies are required. OCT may also have a significant role in optimizing treatment parameters on an individual patient basis. Variable skin thickness, melanin content, and skin temperature must be accounted for when selecting the optimal laser treatment parameters on an individual patient basis. As OCT technology is advanced, clinicians may be able to use this modality to rapidly determine the depth of collagen injury as a function of laser treatment parameters. Finally, OCT may be used to compare and contrast tissue effects of different laser devices.

IV. Conclusion

We have used OCT to monitor wound healing process in an organotypic model of the skin. Our results indicate that OCT can noninvasively monitor changes in collagen structure and thermal damage. This suggests that OCT has potential to be a powerful method for characterization of collagen injury post-laser irradiation and may be a useful tool for evaluation and comparison of NALSR devices.

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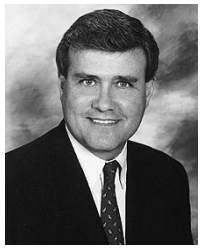

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