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Dynamic study of irradiated artificial skin using OCT

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ABSTRACT

We report a novel application of optical coherence tomography (OCT), to monitor post-laser irradiation collagen injury in skin model. An artificial skin model (RAFT) which closely approximates human skin, was irradiated with a Perovskite laser ($\lambda = 1341$ nm). We investigated dynamic changes in a RAFT after laser irradiation through OCT and compared the results to those of histology. OCT images clearly delineated areas of post-irradiation collagen injury and allowed non-invasive monitoring of the wound healing process. Histology was correlated well with OCT images. OCT has advantages because it is non-invasive and allows serial monitoring at the same site over time. Our study showed that OCT may be a useful tool for determination of optimal parameters for non-ablative laser skin rejuvenation (NALSr) using different devices.

Index Terms — Optical coherence tomography, Non-ablative laser skin rejuvenation (NALSr), Artificial skin model (RAFT), Histology

I. INTRODUCTION

Optical coherence tomography (OCT) is a non-invasive imaging technique capable of performing high-resolution, two-dimensional cross-sectional imaging [1]-[3]. A number of features make OCT attractive for a broad range of applications. Most importantly, OCT is a powerful tool because it enables real-time, in situ visualization of tissue microstructure without the need to excise and process the specimen as in conventional biopsy and histopathology. Consequently, OCT can be a useful method of dynamic measurement.

Direct visualization of tissue structure offers a unique opportunity for evaluation of tissue effects after laser irradiation. During the last decade, non-ablative laser skin rejuvenation has become a popular procedure for treatment of fine facial wrinkles and acne scars [4]-[7]. A variety of lasers and light sources 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 visualize non-invasively 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 are unable to serially image the same site as a function of time [8], [9].

In the present study, we have used OCT to monitor wound healing response 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 7-day period using OCT and conventional histopathology.

II. MATERIALS AND METHODS

2.1 Skin Model Preparation and Laser Irradiation

An artificial skin model (RAFT) was used in our study. An RAFT mimics in vivo human skin in terms of structure, cellular activity and function. The RAFT was composed of human dermal fibroblast cells in a collagen type 1 gel (Discovery Labware, Inc., Bedford, MA) with surface epithelia of human epithelial keratinocytes. We have established 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). A representative histological section of the mature model is shown in Fig. 1. Histology of both the epidermis and dermis of RAFT closely resembles that of human skin. RAFT were irradiated using a Perovskite laser ($\lambda = 1341$ nm, DualisTM by Fotona, Ljubljana, Slovenia). Single pulse parameters were an energy density of 25 or 35 J/cm² and a pulse duration of 20 ms.

Fig. 1. Histology of RAFT: SC, Stratum Corneum; EK, Epidermal Keratinocytes; DF, Dermal Fibroblasts; OEM, Organized Extracellular Matrix.

2.2 OCT Instrumentation

The schematic of the OCT system is presented in Fig. 2. The time delay of light backscattered from skin model 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. 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 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.

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

2.3 Dynamic Measurement

For evaluation of the wound healing process, 8 RAFT specimens were used. Each model was irradiated under identical conditions. One model was imaged daily by OCT over a 7-day period. The imaged area was marked with 100 μm beads so that the exact same position could be relocated every day. Over the 7-day study period, one

specimen was harvested each day for histologic analysis. Samples processed for histopathology were fixed for 24 hours 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

Fig. 3. OCT images of RAFT models: A, non-irradiated sample (13×1.3 mm, $10 \mu\text{m}/\text{pixel}$); B, irradiated sample with energy density of $35 \text{ J}/\text{cm}^2$ (13×1.3 mm, $10 \mu\text{m}/\text{pixel}$); C, magnification images of B ($2\text{mm} \times 1.3$ mm, $10 \mu\text{m}/\text{pixel}$).

OCT image of acutely irradiated sample was showed in Fig. 3. On laser irradiated sample, a layer of intact epidermis is noted at the top of the specimens. An area of a dark region demarks the laser-irradiated area is clearly observed. Since OCT measures backscattering light intensity, the dark region, which corresponds to a reduced OCT signals, indicate that backscattering coefficient is reduced in the laser-irradiated collagen. Collagen in the native state forms super helix bundle and has a large spatial variation of density in 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.

Fig. 4 shows a series of OCT images demonstrating the wound healing response to laser irradiation over a 7-day period. Collagen regenerated gradually over time with significant healing achieved by day 7.

Fig. 4. Wound healing response using OCT of irradiated RAFT over 7 days. The dotted lines outline the irradiated region. Bead marks are clearly shown on both sides of the irradiated area. Size of OCT images are 13×1.3 mm with $10 \mu\text{m}/\text{pixel}$. Sample was irradiated by single pulse ($25\text{J}/\text{cm}^2$).

In Fig. 5 higher magnification views of the OCT images allow closer evaluation of the wound healing response as seen on days 3, 5, and 7. Corresponding histology sections are shown for comparison. On day 3, a relatively large area of injured collagen is noted in the center of the irradiated field. Wound healing is already occurring as evidenced by the presence of fine strands of regenerated collagen and the migration of fibroblasts into the irradiated area. On day 5, more significant collagen regeneration is noted which appears to be initiated from deep and lateral aspects of the specimen where intact collagen survived after laser irradiation. On day 7, 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 devices. There are no at-risk subjects and a wide range of laser parameters can be accurately and rapidly evaluated.

Fig. 5. Comparison between OCT images and histologic sections. OCT image size is 2×1.3 mm with $10 \mu\text{m}/\text{pixel}$.

CONCLUSIONS

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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