In Vivo Monitoring of Microcirculation in Burn Healing Process with Optical Microangiography

Wan Qin, Yuandong Li, Jingang Wang,
Xiaoli Qi, and Ruikang K. Wang*

Department of Bioengineering, University of Washington, Seattle, Washington.

Ruikang K. Wang, PhD

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*Correspondence: Department of Bioengineering, University of Washington, 3720 15th Ave NE, Seattle, WA 98195-5061 (e-mail: wangrk@uw.edu).

Objective: Optical microangiography (OMAG)-based optical coherence tomography is a noninvasive technique capable of imaging functional microvasculature innervating scanned tissue volume. In this study, we utilize OMAG to investigate dynamic changes of microcirculation during the healing process of a burn.

Approach: A soft-contact superficial burn injury was induced on a mouse ear with 1 μ L 70°C hot water. Microangiograms were generated by using OMAG before and after the burn.

Results: Vessel recruitment and remodeling were observed in the healing process. Burn injury reached to the worst extent within the first 24 h and had no expansion thereafter. The interrupted microcirculation in the mouse ear was progressively recovered in the consequent postburn days and completely healed on postburn day 7.

Innovation: OMAG provides a novel way for noninvasive visualization and quantification of vasculature changes over time after burn injuries. The high resolution achieved by the imaging system reveals microvascular details down to capillary level.

Conclusion: Our results demonstrated that OMAG has great potential to improve the understanding of microcirculatory responses to burns and thus benefit the development of effective therapeutics.

INTRODUCTION

AN ESTIMATED 500,000 burn injuries receive medical treatment yearly in the United States.¹ The treatment of burns remains a challenge. No causal therapy, besides the administration of physiologic saline, local disinfection, and symptomatic medications, is known to reduce the tissue damage and accelerate burn healing.² Recent advances in cellular and molecular biology have determined the three phases involved in the healing process of burns: inflammatory phase, proliferative phase, and remodeling phase.^{3,4} Microcirculation plays an important role in all these phases

because it provides a means to regulate the blood flow and tissue perfusion, mediates the delivery of nutrients and oxygen to living tissue, and thus sustains the newly formed granulation tissue.⁵

Most experimental burn studies have employed *in vitro* techniques based on analyzing histological samples.⁴ These *in vitro* models have provided significant information on the microcirculatory pathophysiology of burn wounds. The main drawback of these methods is the inability to correlate structure with function and the recognition of preparation artifacts. Due to the lack of suitable *in vivo* technologies for visualization

of vasculature, studies of dynamic phenomena such as vasoconstriction and dilation, changes in blood flow distribution, thromboembolism, and microcirculatory standstill in the burn wound have been inconclusive. Conventional angiographic imaging technologies, for example, magnetic resonance imaging and computed tomography angiography, are costly and are difficult to provide sufficient spatial and temporal resolutions required for imaging changes in capillary tissue beds.^{6,7} Multiphoton microscopy is capable of high-resolution visualization of capillaries but with a small field of view, limited penetration depth, and the need for exogenous contrast agents.⁸ Photoacoustic microscopy can detect several blood perfusion parameters within small vessels.⁹ However, it is not contact-free and thus possibly complicates the interpretation of blood flow in the contacted tissue region. Laser speckle imaging has also been utilized for *in vivo* investigation of relative changes in blood flow, but it is not a depth-resolved technique and is applicable to only large vessels where the depth axis is roughly averaged.¹⁰ The drawbacks of current imaging modalities limit the investigation of the dynamic response of microcirculation to burn injuries.

Optical microangiography (OMAG), as one of the most significant extensions of conventional optical coherence tomography (OCT) technique, was recently developed for three-dimensional (3D) imaging of dynamic blood perfusion within microcirculatory tissue beds.^{11,12} OMAG is a noninvasive and label-free imaging technique. The contrast of OMAG images is produced by the intrinsic light

scattering from flowing blood cells. With this technique, the sensitivity of 4 $\mu\text{m/s}$ to the blood flow has been reported.¹³ With such a high sensitivity to blood flow, OMAG has been extensively employed to visualize functional blood flow networks innervating microcirculatory tissue beds in small animal models and has also been applied to human skin and eye imaging.^{14,15} In this study, we applied the OMAG technique to investigate the dynamic microcirculation in a mouse ear after a thermal injury was induced on the ear pinna with hot water.

CLINICAL PROBLEM ADDRESSED

With a novel imaging modality, OMAG, we monitored the microcirculation in the healing process of a superficial burn in a mouse ear. The imaging results revealed vessel recruitment and remodeling in the tissue bed after the burn without the need of contrast agents, demonstrating that OMAG could be a promising tool for *in vivo* examination of microcirculation in response to burn injuries.

MATERIALS AND METHODS

The OMAG-based OCT imaging system is schematically shown in Fig. 1, which has been described in detail in Refs.^{16,17} Briefly, a Supercontinuum (SC) Source (SuperK Versa, Koheras A/S, Denmark) was used as the light source to illuminate the system. The output spectrum of the SC source covered from 460 to 2,400 nm and was modulated and reshaped into a Gaussian waveform by an optical filter. The output Gaussian spectrum from the optical filter was

Figure 1. Schematic of optical microangiography (OMAG) imaging system. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 2. Projection view images of vasculature network in the mouse ear obtained with OMAG before and after the burn injury on different days: **(a)** Baseline: before the burn, **(b)** day 0: right after the burn, **(c)** day 1, **(d)** day 3, **(e)** day 5, and **(f)** day 7. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

centered at 820 nm wavelength with a bandwidth of 120 nm. Owing to such a wide bandwidth, a high axial resolution ($\sim 3 \mu\text{m}$) was achieved with the fiber-based spectral domain OCT system. To avoid photodamage in biological tissues, the optical power illuminated on the sample was always kept at $\sim 3 \text{ mW}$. As the fast detector of spectral interferograms, a high-speed spectrometer was built with a complementary metal-oxide semiconductor line-scan camera (4,096 pixels; Basler SPL 4096-140 KM) that is capable of a maximum line-scan rate of 140 kHz. A $10\times$ objective lens with an effective focal length of 18 mm was used as the scanning lens that delivered a lateral resolution of $9 \mu\text{m}$ at full width half maximum level. The 3D imaging was accomplished by a pair of galvanometer scanners that scanned the probe beam on the sample.

To achieve ultra-high sensitive OMAG imaging, a specially designed scanning protocol was applied.¹³ Briefly, in the fast scan (x) direction, each

B-frame (cross-sectional image) was scanned with 400 A-lines. The spatial interval between adjacent A-lines was set at $8.5 \mu\text{m}$ that is slightly smaller than the lateral resolution of the imaging system. In the slow scan (y) direction, 2,000 B-frames in total were captured at 400 locations (5 frames were acquired at each location, rendering a total 400 of equal spaced locations). The spatial interval between adjacent B-frames was $\sim 8.5 \mu\text{m}$ as well, so that the scanning range (field of view) in x - y plane was $3\times 3 \text{ mm}$. When the camera works at its maximum line-scan rate, 140 kHz, the highest frame rate, 280 frames per second can be achieved. In this study, a frame rate of 250 was utilized. At this frame rate, an acquisition time of 8 s was required to finish one 3D imaging, which is short enough to minimize motion artifacts for *in vivo* imaging.

To study the dynamic changes in microcirculation during the process of burn healing, we first

Figure 3. Enlarged blood vessel images of the center region of the burn, region of interest (ROI) 6, and one of the peripheral regions, ROI 2, on different days: (a–f) ROI 6 of baseline, days 0, 1, 3, 5, and 7, respectively; (g–l) ROI 2 of baseline, days 0, 1, 3, 5, and 7, respectively.

established an animal model with superficial scald burn injuries. A widely used burn model is inducing thermal injury on ears of mice or rats with a hot metal bar. However, this model is always accompanied with mechanical trauma at the injury site due to the hard contact of the hot metal tip with the skin, which, besides the burn, can significantly affect the microcirculation and leukocyte behavior.² In our model, we created a soft-contact burn on a mouse ear using a drop of hot water, which prevented the presence of mechanical trauma. All experimental animal procedures were carried out in conformity with the guidelines of the National Institute of Health for care and handling of laboratory animals. The laboratory

animal protocol for this work was approved by the University of Washington Institutional Animal Care and Use Committee.

The experimental procedure was as follows: SKH-1 hairless mice (23–25 g; Charles River, Hollister, CA) were anesthetized with isoflurane (0.2 L/min oxygen and 0.8 L/min air) by a face mask. The mouse ear flap was immobilized and stretched over a fixed platform to minimize the motion resulted from breathing and heartbeat. The body temperature was maintained between 36.7°C and 37.1°C monitored by a rectal thermal probe. As a control, the first OMAG acquisition was taken before the burn. To induce a superficial burn on the ear skin, we used a micropipette to transfer a drop

of hot water ($1\ \mu\text{L}$, 70°C) onto the skin of the pinna. Thirty seconds later, the water on the pinna was dried out, and the second OMAG acquisition was performed to record the immediate response in microcirculation following the burn. This data point was marked as "Day 0." To observe the chronic healing process, subsequent OMAG acquisitions were obtained on postburn days 1, 3, 5, and 7.

RESULTS

Figure 2 shows the microangiograms of the mouse pinna before and after the burn injury, which were acquired with the OMAG-based OCT system. Each microangiogram is a mosaic of six OMAG images ($\sim 3 \times 3\ \text{mm}$) that were captured from different regions of the mouse ear and stitched together to create a large field of view ($5.8 \times 8.6\ \text{mm}$). The microangiograms present the *en face* maximum projection view images of the vasculature network from the mouse ear. Figure 2a was recorded before the burn injury as the baseline. The red circle indicates the area where the hot water was dropped onto. Qualitatively, it can be observed that more blood vessels appear in Fig. 2b, the microangiogram of postburn day 0 (immediately after the burn), compared with the baseline. Since the day 0 image was captured only a few seconds after the baseline, the appearance of new blood vessels was not resulted from angiogenesis but mainly due to capillary recruitment. The new vessels actually preexisted but had no or quite slow blood flow that cannot be detected with OMAG in the baseline (the minimum blood flow velocity required by OMAG is $4\ \mu\text{m/s}$).¹⁵ This phenomenon is different with that of partial-thickness burns because superficial burns hurt only epidermis layer while partial-thickness burn injuries extend to dermis and damage blood vessels. The immediate response in microcirculation after a superficial burn is usually hyperemia in the burn area, while a partial-thickness burn would cause coagulation at the site of the burn and hyperemia in peripheral areas.¹⁸ In the subsequent postburn days, the progressive occlusion and reperfusion processes were monitored (Fig. 2c–f). On the second day postburn (day 1), significant reduction of blood vessels was observed. This is because, in the first 24 h postburn, progressive ischemia resulted from initial venous thrombosis, leading to arteriolar occlusion.¹⁹ The reperfusion started from day 1 and completed on day 7.

To quantitatively analyze the vasculature changes before and after the superficial burn injury, we evaluated vessel density (VD) over areas of interest. The VD is a criterion that is commonly utilized to quantify blood vessel changes. We em-

ployed method of Reif et al. to calculate the VD,²⁰ by which we binarized the OMAG image with an adaptive threshold, counted white pixels that represent the area covered by vessels, and divided it by the total pixel number of the image. The blue boxes in Fig. 2a indicate the six regions of interest (ROIs), including the center region of the burn and five peripheral regions where we evaluated the VD. Two of the ROIs, ROI 6 in the center region of the burn and ROI 2 in the peripheral region, are selected to enlarge to show detailed changes in capillaries before and after the burn on different days, as illustrated in Fig. 3. The calculations of VD over the six ROIs are statistically shown in Fig. 4, demonstrating the trend of the VD change during the healing process of the superficial burn: the burn injury reached to the worst extent within the first 24 h and had no expansion thereafter; the interrupted microcirculation in the mouse ear was progressively recovered in the consequent postburn days and almost completely healed on postburn day 7.

INNOVATION

Microcirculation plays an important and complex role in burn healing. Visualization of dynamic

Figure 4. Mean and standard deviation of vessel area density for the six ROIs determined in Fig. 2a on different postburn days. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

microcirculatory response to burns in tissue is essential for comprehensive understanding of each phase of burn healing and thus beneficial to development of effective therapeutics. OMAG allowed the noninvasive visualization and quantification of vasculature changes over time after the burn injury. The high resolution achieved by the imaging system revealed the microvascular details down to capillary level. Our experimental results demonstrated OMAG-based OCT could be a practical tool for dynamic investigation of burns and tracking microcirculation *in vivo*.

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AUTHOR DISCLOSURE AND GHOSTWRITING

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KEY FINDINGS

- OMAG has excellent potential for clinical investigation and examination of microcirculation in burnt tissue owing to its noninvasive and 3D feature.
- The imaging results suggest that superficial burn injury reaches to the worst extent within the first 24 h and has no expansion thereafter.

ABOUT THE AUTHORS

Ruikang K. Wang, PhD, is currently Professor of Bioengineering and Ophthalmology at the University of Washington, and directs the Biophotonics and Imaging Laboratory. He is an elected fellow of Optical Society of America (OSA), International Society for Optics and Photonics (SPIE) and American Institute for Medical and Biological Engineering (AIMBE). His current research interests include biophotonics and imaging; optical coherence tomography; optical microangiography; photoacoustic imaging; and their applications in neurology, ophthalmology, dermatology, and cancer. **Wan Qin, PhD**, is a Senior Research Fellow in the Department of Bioengineering at University of Washington. **Yuandong Li, BSc**, is the Lab Manager of Biophotonics and Imaging Laboratory. **Jingang Wang, PhD**, and **Xiaoli Qi, PhD**, are also Senior Research Fellows in the Department of Bioengineering at University of Washington.

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Abbreviations and Acronyms

3D = three dimensional
 OCT = optical coherence tomography
 OMAG = optical microangiography
 ROI = region of interest
 SC = supercontinuum
 VD = vessel density