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Authors
Choi, Bernard
Jia, Wangcun
Kelly, Kristen M
et al.

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CHRONIC, WIDE-FIELD OPTICAL IMAGING OF BLOOD FLOW DYNAMICS

Bernard Choi, Departments of Biomedical Engineering and Surgery, Beckman Laser Institute, University of California, Irvine

Wangcun Jia, Department of Surgery, Beckman Laser Institute, University of California, Irvine

Kristen M. Kelly, Departments of Dermatology and Surgery, Beckman Laser Institute, University of California, Irvine

Jennifer Channual, School of Medicine, University of California, Irvine

Justin Lotfi, Department of Surgery, Beckman Laser Institute, University of California, Irvine

INTRODUCTION

Noninvasive blood flow characterization is essential to assess the health status of biological tissue and to evaluate the efficacy of therapies which target the microvasculature. Optimization of laser therapy for disfiguring vascular birthmarks is one specific clinical application. Current treatment protocols involve the use of high-power pulsed laser irradiation with parameters selected to induce selective photocoagulation of the targeted blood vessels[1]. Protocol design is based largely on results from numerical modeling studies[2], which have predictive capability of the laser light distribution within the skin and subsequent photothermal response leading towards selective photocoagulation. However, the biological response of the microvasculature to therapeutic laser intervention remains a poorly-researched field. We hypothesize that the acute photothermal response of the microvasculature is a poor predictor of the chronic response, due to vascular remodeling processes which are not included in current modeling studies. To test this hypothesis, we employ an optical imaging method to assess blood flow dynamics in response to therapeutic intervention.

MATERIALS AND METHODS

Animal model

The data presented herein was acquired from adult male Golden Syrian hamsters. Animal care during and after surgery was in accordance with a protocol approved by the Institutional Animal Care and Use Committee at University of California, Irvine.

Rodent dorsal window chamber

The surgical protocol was a modified version of one described previously[3]. For all steps, aseptic conditions were maintained. Briefly, the animal was anesthetized and the dorsum was shaved and depilatory lotion applied to remove all visible hair. Titanium window frames were attached to both sides of the skinfold, with care taken so the 12-mm-diameter window was placed over the desired location. One full thickness of skin within the window was removed, revealing the subdermal microvasculature of the remaining skin thickness. The remaining layer of skin is approximately 0.5 mm thick, containing with a microvascular network with vessel diameters of roughly 10 to 150 μm. Sterile saline was applied to the exposed skin and a sterile glass coverslip was placed in the frame and secured with a plastic clip.

Laser speckle imaging (LSI)

We have developed a LSI instrument that acquires reflectance images of an object irradiated with low-power laser light[4,5]. Due to the coherent nature of laser light, the images contain speckle patterns. In the absence of moving optical scatterers, such as red blood cells, the patterns are characterized by a high speckle contrast. Scatterer motion results in speckle intensity fluctuation, which in a time-integrated image appears as a loss in speckle contrast (i.e., blurred regions in the image). The degree of speckle contrast loss is representative of a change in local blood flow. With a sliding-window operation, we convert raw speckle images to speckle contrast images. With an assumption of the velocity distribution of red blood cells, we then calculate speckle flow index images.

Our LSI instrument consisted of a coherent light source, camera, and image acquisition system. A fiber-coupled 633-nm laser was the coherent light source. A 12-bit, thermoelectrically cooled, monochrome CCD camera equipped with a macro lens (1:1 magnification) acquired reflectance images from the irradiated object. The lens f/# was selected to match approximately the imaged speckle size with the camera pixel pitch (6.5 μm). Custom LabVIEW software was used to control the camera, acquire each image, and perform image processing. We acquired raw speckle images with an exposure time of 10 ms, which we deemed appropriate to ensure operation within the linear response range of our instrument[5].

General experimental methodology

LSI images were acquired immediately following surgery and then at arbitrarily selected timepoints up to 30 days post-intervention. Color images were first acquired with a 24-bit CCD camera. This camera was then replaced with the 12-bit monochrome camera described above and a sequence of 30 raw speckle images was acquired at exposure times of 10 ms. Each raw speckle image was converted to a speckle flow index (SFI) image using a simplified imaging algorithm (manuscript in preparation). Briefly, the recorded image sequence was converted to speckle contrast images by applying a 7 x 7 sliding window to each image. At each window position, the mean graylevel intensity (<I>) and standard deviation (μ) were determined, and the speckle contrast (C) of the center pixel in the window was computed as $C = \mu/\langle I \rangle$. SFI values were calculated for each pixel as $SFI = (2TC^2)^{-1}$, which is an equation valid for C values in the range of 0 to 0.6.

RESULTS AND DISCUSSION

Our data demonstrate that the biological response is characterized by substantial vascular remodeling and blood flow redistribution over a monitoring period of up to 30 days post-intervention (Figure). We have observed several vascular effects, including vasoconstriction, vasodilation, delayed blood flow changes, and shunting of blood flow to collateral vessels. Furthermore, we have observed vessel repair within the same position as the original vessel, suggesting that the vascular remodeling process may be associated with a "memory", in agreement with published tumor angiogenesis data[6]. In general, our small animal imaging approach allows us to evaluate rapidly novel therapeutic protocols and to identify strategies for protocol refinement to enhance treatment efficacy.

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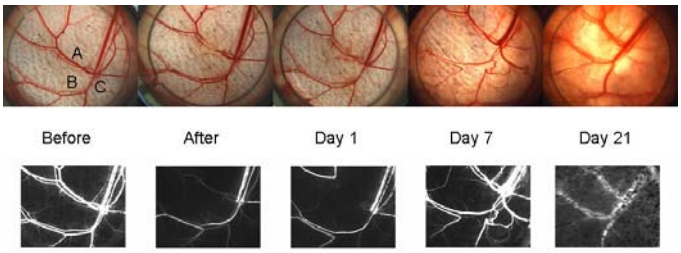

Figure. Representative color reflectance (top row) and SFI images (bottom row) obtained from a hamster dorsal window chamber. In the hamster dorsal skin, the blood vessels are laid out in arteriole-venule pairs. They are easily differentiated by size (arterioles are the thinner of the two). In this experiment, vessel pairs A and B (as denoted in the "Before" image) were irradiated with high-powered laser pulse protocols (laser wavelengths of both 532 and 1064 nm contained within each pulse), with the goal of selective vessel photocoagulation. A substantial acute reduction in blood flow in both vessels at site A and the venule at site B were observed, with vasoconstriction at A. At site B, the blood flow in the venule was absent at Day 1 post-intervention. At day 7 post-intervention, we observed a restoration in blood flow in the vessel pair at site A and shunting of blood flow to collateral vessels at sites B and C (the latter site was not irradiated directly with the laser). At day 21, the collateral vessels have changed shape and position to mimic the pre-intervention microvascular layout (see "Before" image for comparison). Due to tissue regrowth over the microvascular network, the SFI images at day 21 cannot be compared quantitatively to those obtained during preceding days.

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