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Assessment of edema volume in skin upon injury in a mouse ear model with optical coherence tomography

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Abstract

Accurate measurement of edema volume is essential for the investigation of tissue response and recovery following a traumatic injury. The measurements must be noninvasive and repetitive over time so as to monitor tissue response throughout the healing process. Such techniques are particularly necessary for the evaluation of therapeutics that are currently in development to suppress or prevent edema formation. In this study, we propose to use optical coherence tomography (OCT) technique to image and quantify edema in a mouse ear model where the injury is induced by a superficial-thickness burn. Extraction of edema volume is achieved by an attenuation compensation algorithm performed on the three-dimensional OCT images, followed by two segmentation procedures. In addition to edema volume, the segmentation method also enables accurate thickness mapping of edematous tissue, which is an important characteristic of the external symptoms of edema. To the best of our knowledge, this is the first method for noninvasively measuring absolute edema volume.

Keywords

Optical coherence tomography; Optical microangiography; Edema assessment

Introduction

Edema is a condition characterized by the abnormal accumulation of interstitial fluid within injured tissue. It occurs when there is an imbalance of hydraulic and oncotic pressures in the filtration system between the capillary and the interstitial spaces [1–3]. The formation of massive tissue edema is well-recognized as a characteristic of tissue injury or inflammation. Injury-induced edema is responsible for the loss of intravascular fluid resulting in fluid imbalance between the interstitium and the lymphatic outflow system [4–7]. Typically, the edema formation is most rapid in the first few hours after injury, possibly leading to hypovolemia and hypoproteinemia in the absence of large volume resuscitation. Further injury, such as compartment syndrome, may occur as the edema can also cause tissue hypoxia and excessive tissue pressure [8, 9]. There are currently no effective clinical treatments that are able to control the degree of edema [1, 8]. One of the main difficulties

associated with developing such a therapy and in improving our understanding of edema pathophysiology is the lack of an efficient method to noninvasively monitor and accurately measure the edema process.

Currently, the most commonly utilized method in the laboratory for assessing edema is to measure the wet to dry weight of tissue biopsy [10]. However, this method requires careful tissue sampling for correct quantification and is not suitable for clinical assessment due to its invasive nature. Plethysmography is a clinically employed technique that measures edema, particularly in limbs, through perometry, water displacement volumetry, and longitudinal circumferential measurements [11, 12]. However, it provides only a rough estimate and is not capable of providing precise edema measurements. Bioelectrical impedance analysis (BIA) is a technique for estimating body composition and has been applied to edema research in recent years [13, 14]. It can quantify the amount of edema by determining and comparing the electrical impedance of healthy and edematous tissues. Optical techniques have also been introduced as noninvasive means to quantify edema, among which, dichromatic absorptiometry (DA) uses a dual photon source to scan the tissue and records the variable attenuation of the two photon energies to measure the interstitial fluid [15]. DA is sensitive to changes in edema in vivo over time. Nevertheless, similar to the limitation of BIA technique, it reveals only relative changes rather than absolute edema volume. Both DA and BIA require a baseline for comparison, and this has limited their application in clinical studies.

Optical coherence tomography (OCT) is an emerging three-dimensional (3D) imaging modality that enables volumetric deep tissue imaging up to a few millimeters at a micrometer resolution [16, 17]. Optical microangiography (OMAG) is an innovative extension of the OCT technique, providing co-registration capability of both functional micro-vasculature and microstructure of the surrounding tissue bed in 3D [18]. Owing to its noninvasive nature and depth-resolving ability, OMAG has been widely applied to various biomedical and clinical studies [19–25]. Recently, OCT technique has been explored to visualize blood and lymphatic vessels in animal models [26–28] as well as in human skin [29]. The principle of the OCT-based lymphangiography (OLAG) stemmed from the fact that the lymph in lymphatic vessels is optically transparent which enabled the lymphatic vessels to be extracted from the tissue bed by detecting dark-signal entities. The interstitial fluid in edematous tissues is similarly optically transparent and is eventually drained by the lymphatic system. Therefore, the concept of OLAG can be ideally adapted to noninvasive assessment of edema in vivo.

In this study, we propose a semi-automatic segmentation method to accurately measure, for the first time, the absolute volume of edema over time following a tissue injury. The segmentation procedure also enables the quantification of changes in tissue thickness, an important exterior characterization of edema. In our study, the edema is initiated by a superficial partial thickness burn in a mouse ear model that was established in our previous work [30]. OCT imaging is performed before and after the thermal injury. Our segmentation results precisely reveal the significant increase of edema formation in the mouse ear in response to the burn.

Methods

Animal model

SKH-1 hairless mice (23 to 25 g, Charles River, Hollister, CA) were used in the imaging experiment for demonstrating the proposed edema segmentation method. The animal was anesthetized with isoflurane (0.2 L/min oxygen and 0.8 L/min air) by a face mask. Its ear pinna was immobilized on a precision stereotaxic stage to minimize its movement caused by breathing and heartbeats. The body temperature was monitored by a rectal thermal probe and maintained at between 36.7 to 37.1 °C with a feedback heating pad during the imaging.

There is a popular burn model, in which a hot metal bar is used to induce thermal injury on animal skin. In this model, not only thermal injury but also mechanical trauma occurs at the injury site due to the hard contact of the hot metal tip with the skin [31], which may complicate the investigation if we aim to study the burn reaction of the skin. To mitigate this problem, we instead create a superficial partial thickness burn on a mouse ear using a drop of 100 °C water. The soft-contact of the water and the skin would eliminate the risk of mechanical trauma and also prevent the displacement of the sample, facilitating the comparison of the imaging results acquired before and after the burn.

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 used in this study was approved by the University of Washington Institutional Animal Care and Use Committee (UW-IACUC).

Identification of edema in tissue

Images in Fig. 1a, b are two typical cross-sectional OCT images of healthy and edematous mouse ear, respectively, which were acquired using a spectral domain-OCT (SD-OCT) system (see below). In the case of healthy mouse ear, the full thickness is typically 250–300 µm on average. It consists of two full-thickness layers of skin symmetrically separated by articular cartilage, without the presence of sweat glands and subcutaneous fat [32]. The layers of the ear can be clearly distinguished from Fig. 1a, where the yellow arrows point to the epidermis (E), dermis (D), and articular cartilage (AC). The normal tissue is mostly homogeneous and thus generates brighter OCT signal than that of the noise floor. We randomly picked a location in Fig. 1a and plotted the OCT signal (orange dashed line) along its depth to show the intensity distribution of the signal in Fig. 1c. The first 100 µm of the line was above the skin surface, where the signal intensity indicated the level of noise floor of the imaging system. As can be seen in Fig. 1c, signal intensity in the epidermis and dermis regions were always higher than the noise floor. In contrast to the healthy ear, the edematous ear as shown in Fig. 1b was much thicker. We choose another location in Fig. 1b (green dashed line) to plot the OCT signal distribution along the depth in Fig. 1d, which exhibited a low signal region within the dermis layer whose signal strength was close to the noise floor. Since the signal strength is determined by the scattering property of the sample, a low signal close to the noise floor level suggests an optically transparent sampling point. Within the tissue bed, the transparent sampling points likely originate from either lymphatic

vessels or edema, and the latter dominated in this case. Qualitatively, we can also recognize both the lymphatic vessels (LV) and the edema (ED) as dark areas in Fig. 1b.

Imaging and processing procedures

The SD-OCT imaging system used in this study has been described elsewhere in detail [28]. Briefly, a SuperK Versa Supercontinuum Source (Koheras A/S, Birkerød, Denmark) was employed as the OCT light source. The output Gaussian spectrum was centered at 820-nm wavelength with a bandwidth of 120 nm, providing an effective axial resolution of 3 μm in air (2.2 μm in tissue). Interferograms formed by lights between the reference and the sample arms were detected by a home-built spectrometer that consists of a collimating lens, a volume transmission grating, and a complementary metaloxide semiconductor (CMOS) line-scan camera (4,096 pixels, Basler SPL 4096–70 KM, Germany). Scanning with a $\times 10$ objective lens (Thorlabs LSM02, Newton, New Jersey), the system delivered a lateral resolution of $\sim 8 \mu\text{m}$ at full width half maximum (FWHM) level and a sensitivity was $\sim 95 \text{ dB}$ around the zero-delay line, falling off to 75 dB at $\sim 3 \text{ mm}$ position. Incident optical power onto the sample was measured at $\sim 3 \text{ mW}$.

The whole procedure of edema assessment using OCT/ OMAG is illustrated as a flow chart in Fig. 2. First, the mouse ear was imaged with the SD-OCT system using an ultrahigh sensitive OMAG scanning protocol. With this scanning protocol, 400 A-lines were captured in the fast scan (x) direction to compose one B-scan, and 2,000 B-frames were scanned at 400 consecutive locations (five repetitive B-scans at each location) in the slow scan (y) direction. The imaging frame rate was 250 fps, and the acquisition time of an entire 3D dataset was 8 s. The OCT raw data was then processed with an OMAG algorithm to produce two outputs, 3D microvasculature and microstructure. Subsequently, the latter was further processed with an attenuation compensation algorithm followed by two segmentation procedures, which will be detailed in the following section. The final segmentation result was a combination of 3D volumes of the interstitial fluid and the lymphatic vessels. Generally, lymph in the lymphatic vessels comes from the interstitial fluid. It is essentially the portion of the interstitial fluid that has been collected by lymphatic capillaries for drainage. It is therefore reasonable to consider the total volume of interstitial fluid and lymphatic vessels as the edema volume.

Edema segmentation method

Figure 3 illustrates how the edema volume was extracted from OCT microstructure images. Figure 3a is a cross-sectional OCT image of an edematous mouse ear. The light attenuation along the depth was first compensated using an algorithm reported in Ref. [33]. It makes the signal intensity of tissue scatterer relatively uniform at every depth and minimizes the shadow casts that appear below blood vessels. This is necessary because the dark pixels in the areas of deep tissue and shadows may cause false-positive identification of edema. Figure 3b shows the corresponding image after the application of attenuation compensation to the original OCT image. As judged from Fig. 3b, the attenuation compensation algorithm effectively minimized the tailing shadows of blood vessels, improved the visibility of deeper tissue, and enhanced the contrast of the image. Afterwards, a user-guided segmentation method [34] was applied to the compensated image to select a certain layer for further

processing, as shown in Fig. 3c. In this step, irregular boundaries between epidermis, dermis, and articular cartilage were initially defined by two manually sketched lines, which guided our custom-developed software to automatically search and trace the layer boundaries throughout all the cross sections. Consequently, the layer between epidermis and articular cartilage was segmented with the two boundaries [34]. The purpose of this layer segmentation was to exclude non-tissue noise area and the articular cartilage from the region of interest. As is known, water is the most abundant component of articular cartilage, contributing up to 80 % of its wet weight [35]. The selected layer as depicted in Fig. 3c ensured a fully automatic and accurate extraction of edema volume from the region of interest in the next step. Figure 3d shows the final segmentation result of the edema. It was obtained by binarizing the region of interest with an adaptive threshold and isolating the low-intensity pixels with a binary value 1 [27, 28]. Images in Fig. 3e, f are the 3D renderings of the tissue structure of the selected layer and the edema volume extracted from this layer, respectively. By comparing the two 3D images, one can appreciate the edema severity. Moreover, we created an overlay to co-register the 3D tissue structure and edema volume in Fig. 3g. It enabled us to examine the spatial relationship between the edema and surrounding tissue bed.

Results and discussions

OMAG/OCT imaging results

OMAG/OCT imaging of the mouse ear pinna was taken before and after the superficial partial thickness burn, and the imaging results are shown in Fig. 4. At each time point, nine acquisitions were made from different regions of the mouse ear and stitched together to cover a larger field of view (6 mm × 6 mm). To minimize hyper-reflective noise signal from the skin surface, a 5-mm diameter cover-glass was put on the top of the skin. After post-processing of the OCT raw data using the method described above, we obtained the 3D tissue microstructure, microvasculature, and edema volume of the mouse ear, separately. The two images in the left column of Fig. 4 are the imaging results at baseline (before the burn), while the other two images are post-burn results. Images in Fig. 4a, b are top views of 3D overlays of tissue microstructure and microvasculature acquired before and after the burn, respectively. To facilitate better visualization, 3D microstructure volumes were rendered partially transparent for a better view of tissue information beneath the skin surface. The black patterns that appeared outside the cover-glass were caused by hyper-reflective noise. From Fig. 4a (baseline), we can see several major arteriole–venule bundles and rich capillaries in the mouse ear that were clearly visualized within the field of view. After baseline acquisition, a drop of 100 °C hot water was quickly transferred from a beaker, which was under heating by a hot plate and was monitored by a thermometer, onto the pinna skin with a micropipette. Twenty seconds later, the water was gently dried out from the skin with a wiper. The second acquisition was started at 5 min post-burn. Since the full field of view was composed of nine images from consecutive acquisitions, data collection took approximately 4 min. During this time period, the injured mouse ear was undergoing acute immunoreaction, and the physiological condition and morphology of both the tissue bed and the vasculature were continually varying as the tissue continued to respond to its injury. For this reason, the OCT signal of the tissue structure was not uniform among all the nine sub-

images, shown in Fig. 4b, and the stitching borders between sub-images appeared more apparent than those in Fig. 4a. The injury site of the skin was highlighted by a red circle in Fig. 4b. In the center of the burn wound, we observed coagulation in both big vessels and capillaries. This signaled damage to the vascular endothelium, likely leading to increased permeability of blood vessels and thus edema.

Images in Fig. 4c, d are en face maximum intensity projection (MIP) images of an overlay between the microvasculature and edema segment before and after the burn, respectively. The red color represents blood vessels and the cyan color is edema. From Fig. 4c, we can see that at baseline, little fluid was accumulated in the interstitium and most of the cyan parts were pre-collecting lymphatic vessels and initial lymphatic sacs. Following thermal injury, a large amount of interstitial fluid was quickly accumulated, and the lymphatic system was expanded in terms of lymphatic vessel density and diameter, as shown in Fig. 4d. Similar to the red circle in Fig. 4b, the orange dashed circle in Fig. 4d also outlines the injury site. By virtue of the co-registration of tissue structure/ microangiogram and the edema image, we were able to locate the edema distribution readily and investigate the relationship between edema formation and physiological alteration of the surrounding tissue/vasculature. Interestingly, the edema fluid was not distributed symmetrically around the injury site. Instead, edema occurred on the right side of the injury more severe than on the left. To quantitatively analyze this phenomenon, we equally divided the full field of view into 9 sub-regions with the coordinates shown on the top and the left side of Fig. 4. “C” denotes “column”, and “R” means “row”. In this way, the nine sub-regions of the baseline image are marked as R1C1, R1C2, R1C3, R2C1, R2C2, R2C3, R3C1, R3C2, and R3C3; the nine sub-regions of the post-burn image are R1C4, R1C5, R1C6, R2C4, R2C5, R2C6, R3C4, R3C5, and R3C6. We selected three representative regions for quantification: the burn area (R2C2/R2C5), upstream peripheral (R2C3/R2C6), and downstream peripheral (R2C1/R2C4).

Figure 5 schematically illustrates the definitions of the terms, upstream peripheral (UP) and downstream peripheral (DP). In this schematic, the black color represents an inverse binarization of the microangiograms over a merged region of R2C1, R2C2, and R2C3 (most of capillaries are erased for the sake of clarity). The red lines are sketched following arterioles, and the blue lines outline venules. The purple circle indicates the burn injury location where the hot water was dropped at. Branching of arterioles and venules are described with red and blue numbers, respectively, sorted from the order of 1 to 4. The injury site is located at the area with second- and third-order arterioles/venules. As the region on the right side of the burn area covers arteriole–venule bundles with higher order, it is defined as the upstream peripheral (UP) region. On the contrary, vessels on the left have lower order where the region is named downstream peripheral (DP).

Burn edema segmentation

Using the segmentation method described in “Methods” section, we obtained volumetric edema images of the three regions of interest at the time points before and after the burn in Fig. 6a. These images qualitatively reveal changes of edema volume in response to burn injury at different locations. Based on the segmentation results, we can also measure absolute value of edema volume. Since the OMAG scanning protocol utilized in the data

acquisition scanned 400 B-frame locations, each of which was composed of 400 A-lines, and the imaging field of view was $2\text{ mm} \times 2\text{ mm}$, the actual voxel size in tissue was $5\text{ }\mu\text{m} \times 5\text{ }\mu\text{m} \times 2\text{ }\mu\text{m} = 50\text{ }\mu\text{m}^3$. By counting the total voxel number of segmented edema volume, we calculated absolute values of edema volume in the six regions of Fig. 6a and exhibited the results as a bar chart in Fig. 6b. Initially, fluid detected in the healthy mouse ear was mainly in pre-collecting lymphatic vessels and initial lymphatic sacs, yielding negligible edema calculation. The values of edema volume in regions R2C1 (DP), R2C2 (Burn), and R2C3 (UP), were measured to be 4.74, 7.35, and 10.31 nL, respectively. After we induced the thermal injury on the ear, edema volume was significantly increased to 17.99 nL in the DP region, 139.24 nL in the burn region, and 291.66 nL in the UP region.

Thickness mapping

Because the accumulation of edema fluid results in abnormal expansion in interstitial space, an increase in tissue thickness is a typical symptom of edema. Therefore, measurement of tissue thickness, in addition to measuring edema volume, is also clinically valuable for estimating edema severity.

The first step of the segmentation procedure, as illustrated by Fig. 3c, extracts the tissue layer between epidermis and articular cartilage. Meanwhile it records the coordinates of top and bottom borders of this layer. Here we define the distance between the two borders as ear thickness and thus we can quantitatively evaluate thickness changes caused by edema. Note that the ear is constituted by an articular cartilage sandwiched by two full-thickness skin layers. The ear thickness defined here is actually the thickness of a half ear.

Figure 7a shows the thickness map results over the three regions of interest (DP, Burn, and UP) before and after the burn. Thickness values are coded with colors. Color from cool to warm corresponds to thickness from thin to thick. Thickness map enabled a quick and direct recognition of global and local thickness change in the ear. Quantification of ear thickness was also performed and shown in Fig. 7b. At the baseline, ear thickness was measured to be $147.95 \pm 32.59\text{ }\mu\text{m}$ in the DP region, $196.64 \pm 34.12\text{ }\mu\text{m}$ in the Burn region, and $227.35 \pm 52.75\text{ }\mu\text{m}$ in the UP region. After the burn, thicknesses of these three regions were 211.36 ± 64.39 , 441.50 ± 80.84 , and $628.53 \pm 82.34\text{ }\mu\text{m}$, respectively.

Discussion

The injury model utilized in this study to initiate edema formation in the mouse ear was a superficial partial thickness burn. According to literature, edema typically occurs faster in partial-thickness burns than in full-thickness burns [8, 15, 36]. The edema formation rate and the amount of interstitial fluid accumulation are related to the general status of local vascular space and also determined by complex alterations in the properties of capillaries and interstitium [37–39]. Several physical pressures contribute to the force that regulates the exchange of fluid across vascular walls, which can be described with the Starling equation [37]:

$$J_v = K_f(\Delta P - \Delta\pi) \quad (1)$$

where J_v is the fluid filtration flux (the amount of fluid crossing the capillary membrane into the interstitium per unit time); K_f is the filtration coefficient that describes the product of the capillary surface area and the capillary hydraulic conductance; $P = P_c - P_i$ is the hydrostatic pressure gradient, referring to the difference between the capillary hydrostatic pressure (P_c) and the interstitial hydrostatic pressure (P_i); π is the oncotic pressure gradient, proportional to the difference between the capillary oncotic pressure (π_c) and the interstitial oncotic pressure (π_i). As most capillaries in the body are not completely impermeable to all proteins, the reflection coefficient (σ), valued from 0 to 1, is used to correct the π so that $\pi = \sigma(\pi_c - \pi_i)$. The net driving pressure in Eq. (1) is defined as ($P - \pi$) rather than ($P + \pi$) because P pushes fluid out of capillaries while π directs fluid inversely.

In a normal steady state, P is slightly larger than π , resulting in a small positive fluid filtration flux that is balanced by the lymphatic system to maintain a constant interstitial volume [3]. In our experiment, the thermal injury led to fluid imbalance in the mouse ear and lead to acute edema within the first 10 min post-burn. As can be noticed by our imaging and quantification results, edema in the UP region was much more severe compared to the DP region. This phenomenon can be explained through analyzing alterations in the three factors, K_f , P , and π .

1. Filtration coefficient, K_f . K_f is essentially a measure of capillary permeability to volume flow. The value of K_f is a product of the capillary surface area and the capillary hydraulic conductance. When the burn occurred in the mouse ear, a large quantity of oxidants was released immediately, producing oxidant injury which is known to damage endothelial cells and lead to increased capillary permeability [40, 41]. Note that in the region with higher order of arterioles (UP), blood flow was higher, compared to the DP region. Consequently, intraluminal shear force in the UP region was enhanced more than in the DP region, and thus the capillary hydraulic conductance of the UP region was the highest among the three regions. Another fact needs to consider that a portion of the vessels were occluded in the center of the burn, which decreased the capillary surface area in this region.
2. Oncotic pressure gradient, $\pi = \sigma(\pi_c - \pi_i)$. Oncotic pressures, π_c and π_i are generated by colloids in capillaries and interstitium [42]. Due to the burn-induced hypoproteinemia, the concentration of proteins was significantly dropped in capillaries [42, 43]. As a result, π_c decreased after the burn. On the other hand, because of the increasing protein permeability the level of interstitial protein content approached the protein level in the plasma. Therefore, the difference between π_c and π_i fell markedly. In addition, the reflection coefficient, σ , is highly dependent on capillary permeability. It can greatly decrease for an increased capillary permeability [44]. These changes determined smaller oncotic pressure gradients in the three regions compared to the baseline. Since the Burn region had the worst hypoproteinemia, π decreased most in the Burn region.

3. Hydrostatic pressure gradient, $P = P_c - P_i$. Interstitial hydrostatic pressure, P_i is always close to zero in normal skin tissues and is not considered to vary much throughout the interstitial compartments [8]. When stimulated by a thermal injury, P_i has been repeatedly reported to have a negative value from the very beginning of the burn [7, 45]. Capillary hydrostatic pressure, P_c , is determined by vascular resistance and is highest at the arteriolar end of the capillary and lowest at the venular end [46]. Additionally, with altered blood flow, venular pressure changes less than arteriolar pressure due to the cumulative effects of peripheral resistance. Therefore, the hydrostatic pressure of capillaries at the arteriolar end dominates the contribution to edema formation. For the case in this study, capillaries in the UP region were close to the first order of arteriole so that the P_c here was the highest among the three regions, whereas capillaries in the DP region were at the end of the third or fourth order of arterioles and thus had the lowest P_c .

For a better illustration, we summarized the alteration in the physical factors that were responsible for the edema formation as a diagram shown in Fig. 8. An increase or decrease in the value of interest was indicated by up or down arrows with the size of the arrow representing the relative magnitude of the change. The overall changes in K_f , P , and π , as illustrated in Fig. 8, at the three regions resulted in highest edema in the UP and lowest in the DP region. The analysis and explanations above were empirically derived based on literature reports, and a more accurate explanation of the edema distribution would require experimental validation with precise measurements of the physical factors in question.

Conclusion

In this paper, we have proposed a method, for the first time, to noninvasively measure absolute edema volume and demonstrated its feasibility in a mouse ear burn model. The superficial-thickness burn was induced with a drop of 100 °C hot water on the mouse ear pinna. The thermal injury caused severe edema that was observed by an SD-OCT imaging system. To extract the edema volume with the OCT images, we first applied an attenuation compensation algorithm to each cross-sectional image to remove shadows casted by blood vessels and enhance the image contrast. Then we utilized a custom-developed semi-automatic software to segment a desired skin layer of the mouse ear. This operation thereby ensured an accurate extraction of the edema volume from the 3D OCT tissue structure images of the mouse ear, which was achieved with an adaptive binarization threshold. The layer segmentation process also enabled thickness mapping over the full field of view which facilitated visualization of local and global changes in tissue thickness as a typical external symptom of edema. Besides the noninvasive nature and the capability to measure the absolute value of edema volume, the proposed technique has another important advantage in that it can readily localize the edema in the surrounding microvasculature or tissue bed by co-registering the edema image to the corresponding microangiogram or tissue structure image. In this way, it provides the possibility to study the pathophysiology of edema formation within any specific local regions in terms of hydrostatic pressure, oncotic pressure, and capillary permeability. As an example in this paper, we selected three representative regions for assessment. By analyzing the alteration in the physical factors over

the three regions of interest, we attempted to explain their different reaction in the edema formation process, though the conclusion from the analysis requires further experimental validation.

Please note that the proposed edema segmentation method is ideal for mouse ear models because the skin structure of mouse ear is simple and thin, without the presence of fatty tissue and sweat gland. Applicable to other animal models or human skin may require further development and optimization. The penetration depth of our imaging system was relatively shallow (~500 μm). However, edema assessment in a thicker skin model could be possible through the use of an alternative OCT imaging system with stronger penetration capability, e.g., swept-source OCT. Another limitation of this edema assessment method is that it cannot distinguish lymph fluid in lymphatic vessels from fluid in the interstitial compartment. However, in most applications, this would not be an issue since the amount of lymph present in the lymphatic vessels is intimately related to the amount of interstitial fluid present in the tissue, and therefore an important feature to assess while investigating edema formation. We attest that it is reasonable to consider edema volume as the combination of lymph and interstitial fluid.

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Fig. 1. Identification of edema signal in tissue through OCT cross-sectional images. **a** OCT cross section of a normal mouse ear. E: epidermis layer; D: dermis layer; AC: articular cartilage. **b** OCT cross section of an edematous mouse ear. LV: lymphatic vessel; ED: edema. **c** Intensity distribution along the orange dashed line in **a**. **d** Intensity distribution along the green dashed line in **b**

Fig. 2.
Flow chart of edema assessment procedure with OMAG

Fig. 3. Illustration of the procedure to extract edema volume from OCT images. **a** Cross-sectional OCT image of an edematous mouse ear. **b** Corresponding image after performing attenuation compensation. **c** User-guided semiautomatic segmentation result of **b**, remaining the tissue layer between the epidermis and the articular cartilage and removing the rest. **d** Edema image segmented from a binarized image of **c**. **e** 3D rendering of the OCT structure images. **f** 3D rendering of the edema volume. **g** Overlay of **e** and **f** showing the spatial location of the edema within the tissue bed

Fig. 4.

Imaging results of mouse ear pinna before and after the thermal injury. **a** Top view of 3D overlay of the mouse ear microstructure and microvasculature acquired before the burn.

Scale bar: 1 mm. **b** Top view of 3D overlay of the mouse ear microstructure and microvasculature acquired after the burn. *Red dashed circle* indicates the burn injury site.

c. *En face* MIP image of overlay of the microvasculature and the edema segment before the burn.

d. *En face* MIP image of overlay of the microvasculature and the edema segment after the burn.

the burn. *Orange dashed circle* indicates the burn injury site. *Red color*: microvasculature; *cyan color*: edema segment

Fig. 5. Schematic illustration of definition of downstream and upstream peripheral regions. *Purple circle*: burn injury site; *red lines*: arterioles; *blue lines*: venules; *numbers in red*: orders of arterioles; *numbers in blue*: orders of venules. Peripheral region with higher order of arteriole/venule is named upstream peripheral, while lower order of arteriole/venule refers to downstream

Fig. 6. **a** 3D views of edema volume in the three regions of interest before and after the burn. *DP*: downstream peripheral region; *B*: burn region; *UP*: upstream peripheral region. **b** Bar chart of edema volume measurements

Fig. 7.
a Thickness maps of the three regions of interest before and after the burn. *Color bar* correlates color with tissue thickness value in micrometers. **b** Quantitative measurements of ear thickness over the three regions at the two time points

Fig. 8.
Diagram of the changes in physical factors in the three regions of interest after the burn.
Upwards arrow: value increased; *downwards arrow*: value decreased; *bigger arrow*: larger changing value; *smaller arrow*: smaller changing value