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In Vivo Optical Imaging of Revascularization after Brain Trauma in Mice

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Abstract

Revascularization following brain trauma is crucial to the repair process. We used optical micro-angiography (OMAG) to study endogenous revascularization in living mice following brain injury. OMAG is a volumetric optical imaging method capable of *in vivo* mapping of localized blood perfusion within the scanned tissue beds down to capillary level imaging resolution. We demonstrated that OMAG can differentiate revascularization progression between traumatized mice with and without soluble epoxide hydrolase (sEH) gene deletion. The time course of revascularization was determined from serial imaging of the traumatic region in the same mice over a one-month period of rehabilitation. Restoration of blood volume at the lesion site was more pronounced in sEH knockout mice than in wild-type mice as determined by OMAG. These OMAG measurements were confirmed by histology and showed that the sEH knockout effect may be involved in enhancing revascularization. The correlation of OMAG with histology also suggests that OMAG is a useful imaging tool for real-time *in vivo* monitoring of post-traumatic revascularization and for evaluating agents that inhibit or promote endogenous revascularization during the recovery process in small rodents.

Keywords

Traumatic brain injury; cerebral blood flow; revascularization; EETs; soluble epoxide hydrolase; optical imaging

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Disclosure/Conflict of Interests

The authors declare no conflict of interests.

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INTRODUCTION

Revascularization is an endogenous process or a surgical procedure for the provision of new, additional or augmented blood supply in order to restore the impaired circulation of affected organ (Carmeliet, 2002). Other medical terms such as neovascularization (new vessel formation) (Arnold and West, 1991), vascular remodeling (structural rearrangement) (Patan et al., 2001) and vascular reperfusion (reopening of occluded vessels) (Clark et al., 1993) are often used to denote specific forms of revascularization.

Endogenous revascularization occurs in a wide range of pathologic processes. Following an ischemic event, the injured tissue relies on peripheral blood perfusion to survive; and revascularization can play a pivotal role in the design of therapeutic strategies against ischemic injury (Folkman, 1998; Krupinski et al., 1994). In the case of penetrating brain trauma where the layers of the meninges are breached and the brain parenchyma is disrupted by mechanical damage, ultra-structural studies indicate that neovascularization occurs in the traumatized brain of adult rats (Frontczak-Baniewicz and Walski, 2003; Morgan et al., 2007). Previous studies also suggest that therapeutic revascularization after therapeutic treatment may repair damaged and leaky vessels, ameliorate vascular insufficiency, and replenish nutrients to ischemic tissues during inflammation (Risau, 1997). Revascularization may also provide direct beneficial effects on axonal remodeling or neural integrity, indicating a new paradigm for treating neuronal disorders caused by traumatic brain injury (Risau, 1997).

To design an effective treatment, we must have a better understanding of vascular restoration under traumatic neurological conditions. However, no means are currently available that can visualize and quantify the dynamic process of revascularization in the course of post-traumatic rehabilitation. Thus, assessing therapeutic potential based on vascular modulation in brain injury models is difficult, if not impossible. In this regard, methods are required that can provide precise identification and quantification of revascularization and monitor the effects of promising agents on augmenting vascular activity in preclinical studies.

Vascular imaging is indispensable for studying trauma-related revascularization and for evaluating the potential of novel modulators to restore microvascular activity. Currently, microscopic methods – ranging from fluorescence, confocal, and multiphoton microscopy to electron microscopic imaging – are often used to elucidate the structure of blood vessels. Magnetic resonance imaging, positron emission tomography, ultrasonography, and optical imaging provide non-invasive, functionally relevant images of angiogenesis in animals and humans. An ongoing dilemma, however, is that microscopic methods provide their highest resolution on preserved tissue specimens, whereas *in vivo* methods give images of living tissues deep within the body but at much lower resolution and specificity and generally cannot resolve vessels of the microcirculation (McDonald and Choyke, 2003).

To circumvent limitations of non-invasive imaging of endogenous revascularization in small animal models of brain injury, we used high-resolution optical micro-angiography (OMAG) (Wang et al., 2007) to observe the process of revascularization in traumatized mice *in vivo*. OMAG is a recently developed novel imaging technique capable of producing 3D images of dynamic blood perfusion within microcirculatory tissue beds at an imaging depth up to ~2 mm below the surface. As a variation of optical coherence tomography technology (Fercher et al., 2003; Tomlins and Wang, 2005), OMAG produces imaging contrasts via endogenous light scattering from moving particles (eg, flowing blood cells within open vessels); thus, no exogenous contrast agents are necessary. The basic physics behind this type of imaging is the well-known Doppler effect. The light backscattered from moving particles has a beating

frequency that can be used to distinguish scattering signals by moving elements from those by static elements. Briefly, OMAG mathematically maps the backscattered optical signals from moving particles into one image – the blood flow image – while it *simultaneously* maps the backscattered optical signals from static particles into a second image – the microstructural image. In this study, we demonstrated that the imaging resolution of OMAG is sufficient to visualize lesion-induced cerebral endogenous revascularization. Because new functional vasculature (with flowing red blood cells) growing in damaged tissue could be detected, we demonstrated the potential of OMAG to study the therapeutic regulation of revascularization in the mouse brain after trauma.

P450 eicosanoid epoxygenic acids (EETs), which are derived from arachidonic acid, are endogenous bioactive lipid mediators that play important roles in vasodilation (Ellis et al., 1990), promotion of angiogenesis (Zhang and Harder, 2002), and many pathophysiological processes. The beneficial effect of EETs, however, is limited by their metabolism via soluble epoxide hydrolase (sEH) (Iliff and Alkayed, 2009; Morisseau and Hammock, 2005). Targeted deletion of sEH, therefore, inhibits EETs breakdown, causing intracellular accumulation and increased levels of EETs in brain. Previous studies showed that sEH pharmacological inhibitors can significantly protect brain from ischemic injury through a vascular mechanism linked to the reduced hydration of EETs (Zhang et al., 2008). Here, for the first time, we used OMAG to investigate endogenous revascularization for up to four weeks after penetrating brain trauma in live mice with and without sEH gene deletion. Our data demonstrated that sEH gene deletion promotes revascularization earlier and more rapidly in genetically engineered mice than in their wild-type counterparts.

MATERIALS AND METHODS

All experimental animal procedures performed in this study conform to the guidelines of the US National Institutes of Health. The laboratory animal protocol was approved by the Animal Care and Use Committee of Oregon Health & Science University (Portland, OR, USA)

Animal model and Experimental protocol

Three-month-old C57BL/6 male mice weighing 20–30g without (wild type, WT) (n=5) and with targeted deletion of sEH (sEH knockout, sEHKO) (n=5) were subjected to penetrating brain trauma by inducing a traumatic lesion in the cortex through the cranium. A 21-gauge needle was disinfected, mounted on a stereotaxic device (Stoelting Co., IL), and used to puncture a round vertical hole at a point 1.0 mm caudal to bregma, 2.0 mm lateral from the midline suture through the skull, schematically shown in Fig. 1A. Brain tissue damage induced by needle insertion is shown in a typical histological section crossing the center of the injury site (Fig. 1B). The injury depth is ~1.5mm measured from the surface of the parenchyma. All mice were euthanized four weeks after brain trauma.

Optical Micro-angiography System and Measurements

OMAG measurements were performed using the system illustrated in Fig. 1C, similar to the one previously described (Wang and Hurst, 2007). Briefly, a broadband infrared superluminescent diode (SLD) with a central wavelength of 1310 nm and a spectral bandwidth of 56 nm served as the light source. The light from the SLD was coupled into a fiber-based Michelson interferometer and subsequently delivered onto a stationary mirror (the reference arm) and focused into the brain tissue via an objective lens (the sample arm). The light backscattered from the sample and reflected from the reference mirror were recombined and then recorded by a home-built high-speed spectrometer that employed a line scan infrared InGaAs array detector. The system had an imaging resolution of $16 \times 16 \times 8$

μm^3 at x-y-z imaging direction and an imaging depth of ~ 2 mm in brain tissue. The minimum detectable blood flow velocity of the system was $\sim 160 \mu\text{m/s}$. To achieve 3D imaging, a pair of X-Y galvo-scanners were used to raster-scan the focused beam across the tissue, with one scanner for the X-direction (lateral) scan over ~ 2.5 mm of the sample and the other for the Y-direction (elevational) scan also over ~ 2.5 mm of the sample. The imaging rate in this study was set at 20 frames (X-scan) per second. The data cube of each 3D image was composed of 500 discrete frames, which took ~ 25 seconds to acquire.

The animal head was shaved and depilated before OMAG imaging. During the imaging, the animal was immobilized on a custom-made stereotaxic stage and was lightly anesthetized with isoflurane (0.2 L/min O_2 , 0.8 L/min air). The body temperature was kept at 35.5 – 36.5°C with a warming blanket and monitored by a rectal thermal probe throughout the experiment. An incision of ~ 1 cm was made along the sagittal suture, and the frontal parietal and interparietal bones were exposed by pulling the skin to the sides. The animal was then positioned under the OMAG scanning probe. In order to acquire the blood perfusion images over a large area of the cortex, the scan was performed clockwise, which resulted in six OMAG images covering the area between the anterior coronal suture and the posterior coronal suture. In this study, the total acquisition time for six images was ~ 8 minutes. A volume segmentation algorithm (Wang and Hurst, 2007) was then applied to each OMAG image to isolate the blood flow signals within the cortex. After stitching the six images together and cropping them, the final OMAG image represented the cerebral blood perfusion over an area of $\sim 4.2 \times 7.2 \text{ mm}^2$ in the mouse cortex. If necessary, maximum amplitude projection was used to project the volumetric blood flow signals onto the X-Y plane to give a 2D view of blood vessel networks, ie, the projection image. Before inducing brain trauma, a control OMAG image was acquired as the baseline. After the trauma surgery, the animal was sutured, disinfected, and given antibiotics and then returned to the cage for rehabilitation. A series of OMAG images were taken at 30 minutes, 24 hours, and weekly up to 4 weeks after brain trauma for all animals.

Histological Evaluation

After the last OMAG images were taken on day 30 after brain trauma, anesthetized mice were transcardially perfused with 0.9% saline for 2 minutes, followed by 4% ice-cold paraformaldehyde. The brains were removed together with the intact cranium and postfixed in 4% paraformaldehyde at 4°C overnight. Blocks of 3-mm-thick slices of brain tissue were then cut, decalcified in 10% formic acid, processed, and embedded in paraffin. Six- micron sections were cut on a sliding microtome (Leica 2155). After drying, the sections were deparaffinized and rehydrated through graded ethanol. Coronal sections were first stained with Hematoxylin and Eosin (H&E) to detect all types of vessels and then stained by CD34 immunohistochemical staining to identify the newly formed vessels around the wound site (Fina et al., 1990). The primary antibody (rabbit anti-CD34, 1:2000) was bridged with a secondary goat anti-rabbit biotinylated antibody (Amersham) and avidin-biotin-peroxidase complex (Vectastain Elite kit, Burlingame, CA), and then visualized with 3, 3'-diaminobenzidine. Negative controls were performed by replacing the primary antibody with non-immune rabbit serum. Each coronal section stained by H&E or CD34 was digitized under a 10x or 40x objective (Nikon), and traumatic areas were identified and compared to the homologous areas in the contralateral side.

Data and Statistical Analysis

The extent of endogenous revascularization or recovered blood supply in the trauma zone was evaluated based on the total volume of blood flow in injured tissue that was identified from the OMAG 3D flow data for each animal. OMAG observations are expressed as mean values with standard deviation (S.D.). Two-way mixed-model analysis of variation

(ANOVA) was performed on the mean blood volume at injury site between WT and sEHKO groups and between different time points to determine whether there is any statistical difference ($p < 0.05$). Depending on the variances of the data, post-hoc test (Tukey) was conducted to determine where the differences were.

RESULTS

OMAG imaging results provide evidence of revascularization after trauma in both WT and sEHKO mice. Two sets of the projection images of cerebrovascular perfusion from representative WT and sEHKO animals are shown in Fig. 2. Because revascularization can now be assessed without sacrificing the animal, it is possible to determine a time course for individual animals. Figure 2 shows sequential intravital images of revascularization during rehabilitation following trauma in a single WT (Fig. 2A) and sEHKO (Fig. 2B) mouse. Before revascularization, the blood flow is totally absent as shown by the black hole in Figs. 2A1, A2, B1, and B2, ie, OMAG detected no blood flow signal. This is due to damage to the vessel walls and hemostasis after bleeding at the trauma site. New blood flow signals within the trauma region indicate the formation of new functional vessels. In the WT mouse, this revascularization process first appeared by week 2 and continued to increase until week 3; whereas in the sEHKO mouse, new blood flow signals were clearly visible by week 1 and continued to increase and connect with established vasculature at least until week 4 after trauma. It should be noted that some veins proximal to the injury site were activated after trauma in both WT and sEHKO mice, expressed by the increased flow intensity and vessel diameter in OMAG images. Their functions were probably related to draining excessive fluid caused by post-traumatic edema and promoting the growth of new microvessels in the avascular area. In the WT mouse, a draining vein only responded to the injury at day 1 (green arrow in Fig. 2A2). However, in the sEHKO mouse, one branch of a draining vein was immediately promoted at 30 minutes after trauma (yellow arrow in Fig. 2B1), and its elevated function was later taken over by another branch of the same draining vein (green arrow in Fig. 2B2) from week 1 to week 4, probably due to its position priority.

The cross-sectional OMAG maps in Fig. 3 detect the precise location of revascularization and exhibit typical evolution patterns of revascularization for the same WT and sEHKO mice imaged in Fig. 2. In the representative WT mouse, the cortical vessels were destroyed by the penetrating injury; and early revascularization occurred on the scanned layer of the brain cortex at week 2. The remodeled vessels were present and extruded into the wound site from week 2 to week 3 and then stopped and regressed by week 4 after trauma. In the representative sEHKO mouse, initial revascularization occurred on the left side of the trauma site before week 1. The vessel density was elevated rapidly and peaked at week 4, nearly filling the whole lesion.

OMAG maps in both Fig. 2 and Fig. 3 comprise the cross-sectional optical images of revascularization development with semi-quantification of the time course of vascular morphogenesis in WT and sEHKO mouse brains. Supportive 3D data from the same representative WT and sEHKO mice are shown in Fig. 4 and exhibit significant differences in new established circulation (green) between WT and sEHKO at week 4 after trauma.

The well-resolved, high contrast 3D OMAG images afforded by light scattering from moving elements in the newly formed blood vessels also allow quantitative measurements of revascularization *in vivo* (Fig. 5). The number of voxels in the same chosen volume at the trauma site from each animal was calculated, multiplied by the voxel dimension and reported as the blood volume due to revascularization. After they are normalized by physical volume of injured tissue, the mean value with the SD among the animals examined was then determined. Within 4 weeks after trauma, the restored circulation detected by OMAG

imaging in sEHKO animals increased markedly and reached a maximum at week 4. In contrast, the blood volume due to revascularization in WT animals increased gradually and peaked at week 3 after trauma. Figure 5 shows that this parameter is significantly different between the sEHKO and WT groups from week 3 to week 4 after injury ($P < 0.03$). Thus, by using OMAG, post trauma revascularization can be quantified without sacrificing the animal.

OMAG data for detecting revascularization in this study were confirmed by histological evaluation 4 weeks after trauma using H&E staining, as shown in Fig. 6(A–E). H&E-stained slices (10x) crossing the center of the trauma site from both WT mice (Fig. 6A) and sEHKO mice (Fig. 6B) demonstrate revascularization, denoted by arrows in the enlarged images (Figs. 6C and 6D) where vessels are highly intertwined with fibrous tissue. The comparison between Figs. 6C and 6D also demonstrates that revascularization is enhanced by sEH gene deletion, which is consistent with OMAG results. CD34 immunohistochemical staining was used to differentiate neovascularization from vascular remodeling or vascular reperfusion. The monoclonal antibody against CD34 reacts with endothelium of arteries and venules, and has been found to stain more intensely capillary endothelium (Fina et al., 1990). It has been used for the diagnosis of vascular tumors and detection of small vessel proliferation representing neovascularization (Mazur et al., 2004; Schaerer et al., 2000). In this study, the CD34 staining results from sEHKO mice showed that the vessels localized at the injury site are partially sensitive to CD34 staining, ie, newly formed vessels (denoted by arrows in Fig. 6G) are intensely stained while remodeled or reperfused vessels (denoted by arrow heads in Fig. 6G) are immune-negatively expressed. In addition, at the parenchymal defects (Fig. 6H), CD34 is densely expressed on new microvessels (denoted by arrows), which are also visualized by H&E staining in Fig. 6E. It should be noted that histological studies show the cortical lesion induced by needle was healed and integrated into the healthy unaffected brain tissue after four-week recovery and repaired tissue with newly appearing vasculature further extruded into the extracortical lesion, suggesting such vascular neoformation detected by OMAG either within or above cortical defects are partially originating from the new vessels growing inside the cortex.

DISCUSSION

Because OMAG permits serial monitoring of microcirculation on the same experimental animal over time, it may be particularly useful for *in vivo* evaluation of evolutionary revascularization observed following brain trauma. Furthermore, OMAG may prove useful in evaluating the effectiveness of therapeutic interventions on revascularization. In our study, by showing the temporal profiles of cerebral blood perfusion at the injury site, the evolution of revascularization could be differentiated between WT and treated mice; and by providing the quantitative information on the evolution of revascularization, the effects of treatment could be further analyzed.

OMAG is a new optical angiography technology that provides markedly improved resolution over the previous *in vivo* vascular imaging systems. For example, the optical methods, laser speckle imaging and scanning laser Doppler imaging (Briers, 2001), have recently been reported to map cerebral vascular structure in small animals, but are of limited use in delineating microvascularization. In practice, the lower limit of flow imaging of OMAG is determined by the optical heterogeneous properties of the sample. For detecting the microvascular flow that has lower velocity, the OMAG imaging speed may need to be reduced in order to increase the dwell time of the probe beam on the slow flows, which would inevitably increase the imaging time required to obtain the necessary blood flow images. Nevertheless, our studies demonstrated that the current OMAG system has sufficient resolution to distinguish revascularization in the brains of mice that were sedated

and restrained during imaging. Thus, assessment of the restored blood flow and, presumably, the patterns of both non-capillary and capillary flow in real time are possible without sacrificing the animal, as would be required in other types of structural imaging studies. Comparing OMAG images between WT (Fig. 2A) and sEHKO (Fig. 2B) animals, revascularization began to mature in 1 week after trauma in treated animals and was accelerated compared to WT mice, in which revascularization began in week 2 after trauma. These data indicate that the sEH knockout mice may evoke early and rapid revascularization in response to traumatic brain injury.

Furthermore, OMAG is capable of resolving both the cortical structures and blood perfusion at depths of ~2 mm through the intact cranium of living mice or small rodents, a penetration depth that cannot be achieved with confocal microscopy. The localized signals from flowing blood may be combined with signals from static structures to capture a complete view of the orientation of blood vessels within the tissue. Thus, we expect that the OMAG technology will be useful not only in providing serial revascularization images, but also in localizing the sites of revascularization in living animals. In this work, 2D OMAG flow images fused with structure images (Fig. 3) clearly identified the dynamic position of endogenous revascularization in two types of traumatized animals over a one-month period of rehabilitation. 3D OMAG flow images around the wound site at week 4 (Fig. 4) compared the recovered functional vasculature in detail between WT and sEHKO mice, as confirmed by H&E histological evaluation (Fig. 6). From the histology studies, we are convinced by CD34 immunoreactive images in both extracortical and cortical defects that new endothelial cells were recruited to form new functional blood vessels after brain injury. We note, then, that the restored cerebral perfusion imaged by OMAG can be attributed to new vessel growth as well as to remodeling of undamaged vessels or reperfusion of blocked vessels.

In addition to *in vivo* volumetric imaging of revascularization after trauma, OMAG can be used to quantify the restored blood flow volume by measuring the number of signals from flowing particles, ie, red blood cells in newly formed vessels. In our study, these quantitative values distinguished the time course of revascularization between WT and sEHKO animals (Fig. 5). The results indicated that revascularization in mice with sEH gene deletion accelerated from week 1 to week 4 after trauma, while revascularization in WT mice was observed at week 2 and slowly developed until week 3 after trauma. The coupling between revascularization and neurogenesis has been demonstrated by previous studies (Carmeliet, 2003; Leventhal et al., 1999; Palmer et al., 2000). Furthermore, revascularization after trauma may alleviate neuronal damage by improving local blood flow and metabolite delivery to the nutrient-deprived neurons (Frontczak-Baniewicz and Walski, 2003). However, whether the noted accelerated revascularization and the extended time window of revascularization in mice with sEH gene deletion aides axonal remodeling or neural integrity deserves further investigation.

In addition, as a non-invasive imaging method, OMAG allows visualization of the dynamic fluctuation in global or localized blood perfusion as well as accurate localization of individual vascular activities that are likely related to pathological revascularization as a consequence of trauma. The serial imaging of our two groups of animals revealed that the enhanced revascularization response in sEHKO mice may relate to vascular regulation from a proximal draining vein that may be associated with proper disposal within the revascularization environment. For the WT animals, no apparent draining vein connected to the trauma region was found after day 1, despite a tendency toward recovery of vascular function; and revascularization remained more depressed in WT after week 3 compared with sEHKO mice, indicating that sEHKO mice have better potential for promoting revascularization. Somewhat surprisingly, the whole-head hemodynamic activity in animals with and without gene deletion dropped markedly at some time points, for example, during

the emergence of revascularization (Figs. 2A4, B3). This vascular depression may be involved in regulating vascular plasticity during this early and vulnerable phase of revascularization. Unlike the draining vein, the fluctuation in cerebral blood perfusion is an unsteady phenomenon in the current animals examined. However, OMAG is undoubtedly a useful tool to investigate this phenomenon on revascularization following pathological conditions.

The current OMAG system is only sensitive to blood flow that is faster than $\sim 160 \mu\text{m/s}$, which is limited by the optical heterogamous property of the tissue as well as the detection unit in the system, ie, the speed of the camera used. However, the flow velocity in capillaries ranges between 50 and 1000 $\mu\text{m/s}$ (Lipowsky, 2005), and is even slower under pathological conditions, such as the current study. Therefore, if the investigation focuses on the initial event of revascularization following trauma, observation of the generation of precursor microvessels will possibly be inhibited, given that this beginning event has a flow velocity slower than 160 $\mu\text{m/s}$. Future studies, then, will focus on developing an ultra-high sensitive OMAG system based on CMOS (Complementary metal–oxide–semiconductor) detectors, so that capillary blood flows within injured tissue regions can be detected and imaged. The high speed afforded by a CMOS camera will not only increase the OMAG imaging speed, but will also facilitate the design of flexible optical beam scanning patterns. These will minimize the effect of the optical heterogeneous property of tissue on the minimal detectable flow velocity, so that the OMAG system will be sensitive to the precursor microvessels within injured tissue regions.

CONCLUSION

The present study demonstrated that OMAG is a useful imaging tool capable of serial imaging of cerebral endogenous revascularization after brain trauma in mice *in vivo*. Using OMAG, we have shown that the differences in revascularization between WT and sEHKO animals can be identified. In contrast to the WT mouse, revascularization detected by OMAG was significantly accelerated after trauma in the sEHKO mouse, indicating that sEH gene deletion may play a role in protecting the endogenous vasodilator EETs. Because of its capability to image blood perfusion with high resolution, OMAG may prove to be a practical tool for tracking revascularization and its vascular regulation following trauma. Without the necessity to sacrifice the animal, we anticipate that OMAG will enhance the use of small rodent models to study novel anti-traumatic therapeutics and repair strategies that are designed to manipulate revascularization for brain injury and other brain disorders.

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Fig. 1.

(A) shows the mouse skin window created for OMAG imaging, where a penetrating brain trauma (shown by a pink dot) was introduced at a region 1.0mm caudal to bregma and 2.0mm lateral from the sagittal suture through the skull. SS, sagittal suture; CS, coronal suture; B, bregma; L, lambda. In (B), brain tissue damage is shown by a typical histological section across the center of the injury site. The injury depth is around 1.5mm measured from the surface of the parenchyma. (C) is the schematic of the OMAG system used to collect the 3D micro-angiography of traumatic brain tissue in small animal model in vivo. CCD represents the charge coupled device, PC the polarization controller.

Fig. 2.

In representative WT (A) and sEHKO (B) mice, OMAG projection images of cerebrovascular perfusion tracked revascularization at injured regions (green boxes) during trauma rehabilitation (A0&B0, baseline; A1&B1, 30 minutes; A2&B2, day 1; A3-A6&B3-B6, week 1- week4). All images except baseline only show ipsilateral sides. For this WT mouse, revascularization first appeared by week 2, and kept growing until week 3; a draining vein (green arrow in A2) responded only at day 1. For this sEHKO mouse, the recovered blood supply was visible by week 1, and continued to increase at least until week 4; a draining vein (yellow arrow in B1) responding to traumatic environment was substituted by another branch (green arrow in B2). The white bar is 1 mm.

Fig. 3.

In representative WT (A) and sEHKO (B) mice, the cross-sectional (x-z) OMAG flow images (red color) fused with OMAG structure images (grey color) detect the precise location of revascularization in trauma zone (pink shadow). Newly formed blood flow migrated into the lesion and nearly filled the wound site in the process of recovery (A0&B0, baseline; A1&B1, day 1; A2-A5&B2-B5, week 1- week4) which is more apparent in sEHKO mouse. These OMAG fused maps also exhibit typical evolution patterns of restored circulation that has described in Fig. 2. The white bar is 500µm.

Fig. 4. 3D OMAG images ($2.2 \times 2.2 \times 2.2$ mm) at week 4 (the last imaging point) show revascularization (green color) situated in the wound site and surrounded by the undamaged functional vessels (yellow color) in representative WT (A) and sEHKO (B) mice. Restored microvessels are clearly seen in both types of mice, but more revascularization is present in the sEHKO mouse.

Fig. 5.

The time course and quantification of restored blood volume (mean $\pm$ s.d.) are determined by the OMAG method and are compared between WT and sEHKO mice. The data is obtained from five pairs of animals. Statistically, revascularization was significantly enhanced by sEH gene deletion in the traumatized mice with the greatest differences occurring between weeks 3 and 4 ($p < 0.03$).

Fig. 6. H&E staining shows revascularization after brain injury at week 4 for representative WT (A) and sEHKO (B) mice, indicated by arrows in the zoomed images (C) and (D), which correspond to the boxes between skull (s) and cortex (c) in (A) and (B) under 10x magnification. CD34 immunoreactive images from sEHKO mice are used to detect neovascularization, denoted by arrows in the enlarged images of the extracortical (G) and cortical (H) sites, which correspond to the boxes in (F) under 10x magnification. The CD34 immuno-negative vessels, denoted by arrow heads in (G), indicate that vascular remodeling or reperfusion is also involved in trauma-induced revascularization, which was also detected by OMAG. The black bar in (A), (B), and (F) is 500 μ m.