Contrast-Enhanced Ultrasound for Assessment of Local Hemodynamic Changes Following a Rodent Contusion Spinal Cord Injury

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ABSTRACT Introduction: Severe trauma to the spinal cord leads to a near complete loss of blood flow at the injury site along with significant hypoperfusion of adjacent tissues. Characterization and monitoring of local tissue hypoperfusion is currently not possible in clinical practice because available imaging techniques do not allow for assessment of blood flow with sufficient spatial and temporal resolutions. The objective of the current study was to determine whether ultrafast contrast-enhanced ultrasound (CEUS) imaging could be used to visualize and quantify acute hemodynamic changes in a rat traumatic spinal cord injury (SCI) model. Materials and methods: We used novel ultrasound acquisition and processing methods that allowed for measurements of local tissue perfusion as well as for assessment of structural and functional integrity of spinal vasculature. Results: CEUS imaging showed that traumatic SCI results in (1) an area with significant loss of perfusion, which increased during the first hour after injury, (2) structural alterations of the spinal cord vasculature, and (3) significant slowing of arterial blood flow velocities around the injury epicenter. Conclusion: We conclude that CEUS has the spatial and temporal sensitivity and resolution to visualize local tissue perfusion and vessel architecture, which maybe useful clinically to determine injury extent and severity in patients with SCI.

INTRODUCTION

The incidence for combat-related spine trauma has substantially increased during recent armed conflicts: from ∼1% during the Korean and Vietnam wars to 5.5–7.4% during operation Iraqi Freedom and Operation Enduring Freedom.[1–3] Taking service members and civilians together, there are ∼5.4 million Americans living with paralysis caused by injuries to the spinal cord.[4] Traumatic spinal cord injury (tSCI) often leads to a debilitating loss of sensory and motor function, autonomic dysfunction, and chronic pain. Currently, there are no imaging methods that can accurately assess irreversible damage to the spinal cord immediately after injury. Development of imaging techniques that can evaluate the degree of irreversible tissue damage acutely after injury can significantly impact acute clinical management, prognosis, and appropriate enrollment in clinical SCI trials.

It is well documented that trauma to the spinal cord leads to a near complete loss of local blood flow at the site of injury along with significant loss of flow to surrounding tissue.[5,6] The loss of blood flow in this surrounding tissue contributes to secondary injury including inflammation, swelling, and progressive cell death. Neuroprotective treatment strategies seek to limit the effects of secondary injury. Evaluating local tissue perfusion and blood flow may yield biomarkers that can predict injury severity and identify specific spinal cord tissue at risk for secondary injury. However, current techniques for monitoring the temporal and spatial patterns of blood flow in the injured spinal cord are limited. Here, we demonstrate the use of a novel approach for an intraoperative tool that allows for visualization of tissue perfusion and local blood flow changes in a rat injury model utilizing ultrafast contrast-enhanced ultrasound (CEUS) imaging. Our novel ultrafast CEUS provides high-resolution images and real-time visualization of hemodynamic changes in and around the injury epicenter after tSCI. This approach allows assessment of local blood perfusion, patent vessel architecture, and blood flow velocities within larger spinal cord vessels.[7] In the current study, we find that a contusion injury of the spinal cord results in a significantly hypoperfused lesion area and disruption of the local spinal vessel architecture. Moreover, the area of hypoperfusion expands within the first hour after injury, which may indicate that loss of perfusion might be partially reversible. These data suggest that CEUS-based imaging shows promise as a means of evaluating local spinal cord blood flow in real time.

METHODS

Rodent SCI Model

Surgical procedures were performed according to an approved protocol following all appropriate guidelines from the University of Washington Institutional Animal Care and Use

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Committee (IACUC Protocol # 4362-01). Female Long Evans rats ($n = 6$, 250–300 g at the time of experiment; Envigo, Indianapolis, Indiana) were utilized for our experiments. The rats were anesthetized using isoflurane (5% to induce and 2.5–3% to maintain) and the area overlying the thoracic vertebral region was shaved, cleaned, and sterilized. A subcutaneous injection of bupivacaine and lidocaine (1.5 mg/kg) was given prior to a longitudinal incision made overlying the T5–T11 area using a #15 scalpel blade. The animals received perioperative subcutaneous lactated Ringer solution (10 mL/kg/h), gentamicin (5 mg/kg), and buprenorophine (0.05 mg/kg). A 24-gauge tail vein catheter was established and attached to a three-way stopcock for the introduction of microbubbles (Definity; Lantheus, New Jersey) and subsequent saline (0.9% sodium chloride) flushes. After subperiostal dissection of paraspinal muscles, a laminectomy was performed to expose the spinal cord from T7 to T9. Animals were suspended at the T7 and T9 spinous process for the contusion injury. A 200-kDyn contusion injury was produced at T8 using an Infinite Horizon spinal cord contusion device (Precision Systems and Instrumentation, LLC, Fairfax Station, Virginia).

Ultrasound Imaging Technique

For ultrasound imaging, the Vantage ultrasound research system (Verasonics, Seattle, USA) combined with a 15-MHz transducer (Vermon, Tours, France) was utilized. For each CEUS acquisition, a bolus of 0.15 mL of microbubbles was injected intravenously followed by a 0.2-mL saline flush. Conventional focused nonlinear contrast sagittal images were acquired over the entirety of a bolus for time intensity analysis. A nonlinear Doppler acquisition and processing was used to detect and separate the nonlinear signals from the lower velocity microbubbles in the microcirculation from higher velocity microbubbles in the larger vasculature.[8–10]

Area Calculation for Perfusion Deficit

All ultrasound images were imported into ImageJ.[11] Perfusion images acquired at midline were used to obtain area of hypoperfusion (<80% of control) using the freehand selection tool.

Vessel Angle Measurements

Images of the larger vasculature, containing higher velocity blood flow, were generated from our nonlinear Doppler CEUS acquisitions. Midline images from each rat and from each time point—preinjury, immediately postinjury, 1 h postinjury—were opened in ImageJ. The angle made between the ventral edge of the spinal cord and vessels extending toward the central canal were measured with the angle measurement tool in ImageJ. Eight to 12 ventral vessels from each time point was measured to create a mean estimate of the change in angle following injury for vessels both rostral and caudal to the injury.

Velocity Estimates

The mean velocity of microbubbles in the larger vasculature was estimated over several cardiac cycles. From mean velocity images, the mean velocity of a region of interest (30×170 μm) in the center of a vessel was calculated in the following vessels for each rat. Microbubble velocities were estimated before injury in ventral arterial and venous vessels. The identification of arterial and venous vessels was based on the pulsatility and direction of flow. Following injury, ventral arterial velocities were estimated in a spinal segment 0.25 mm rostral to the injury, the center of the injury, and segments 0.25 mm caudal to the injury. Ventral venous velocities were measured in spinal segments 0.25 mm rostral and caudal to the injury. Venous vessels were not reliably visualized in the injury center.

Sample Size Estimation and Statistical Analysis

From the mean and variances of the CEUS vascular features (ie, hypoperfusion area, vessel angle, and velocity changes) found in our initial experiments,[7] we estimated six animals would safely ensure our ability to determine significant statistical differences ($p < 0.05$) with 80% power.[12] The objective of the current study was to determine whether ultrafast CEUS imaging could be used to visualize and quantify acute hemodynamic changes after acute SCI. Continuous variables are depicted as means ± standard error of the mean. GraphPad Prism was used for statistical calculations.

RESULTS

Ultrafast CEUS was used to obtain mid-sagittal images of the rat thoracic spinal cord before and after a contusion injury through the acoustic window created by the laminectomy (Fig. 1).

The CEUS perfusion images were obtained by separating the faster flowing contrast agent signals of the macrocirculation from slower moving contrast agent signal seen in the microcirculation.[8–10] Tissue perfusion images of the intact spinal cord showed higher blood perfusion in gray matter compared to white matter, which was expected given greater vascularity of the grey matter (Fig. 1a–c). Immediately following a contusion injury of the thoracic spinal cord, there was an area of significant hypoperfusion detected in the injury center (2.75 ± 0.26 mm^2) in all animals examined (Fig. 2). Interestingly, at 1 hour after injury, the area of hypoperfusion enlarged significantly by 41.9% to an area of 3.90 ± 0.27 mm^2 ($p < 0.03$).

The CEUS angiogram images revealed contrast agent signal within the spinal macrocirculation. Using this mode, we were able to visualize spinal vascular architecture in the intact spinal cord (Fig. 1d–f). Imaging in the mid-sagittal plane allowed for visualization of anterior spinal artery (ASA) ventrally with the branching off central sulcal arteries (CSAs). Immediately after contusion injury, there was a dramatic loss

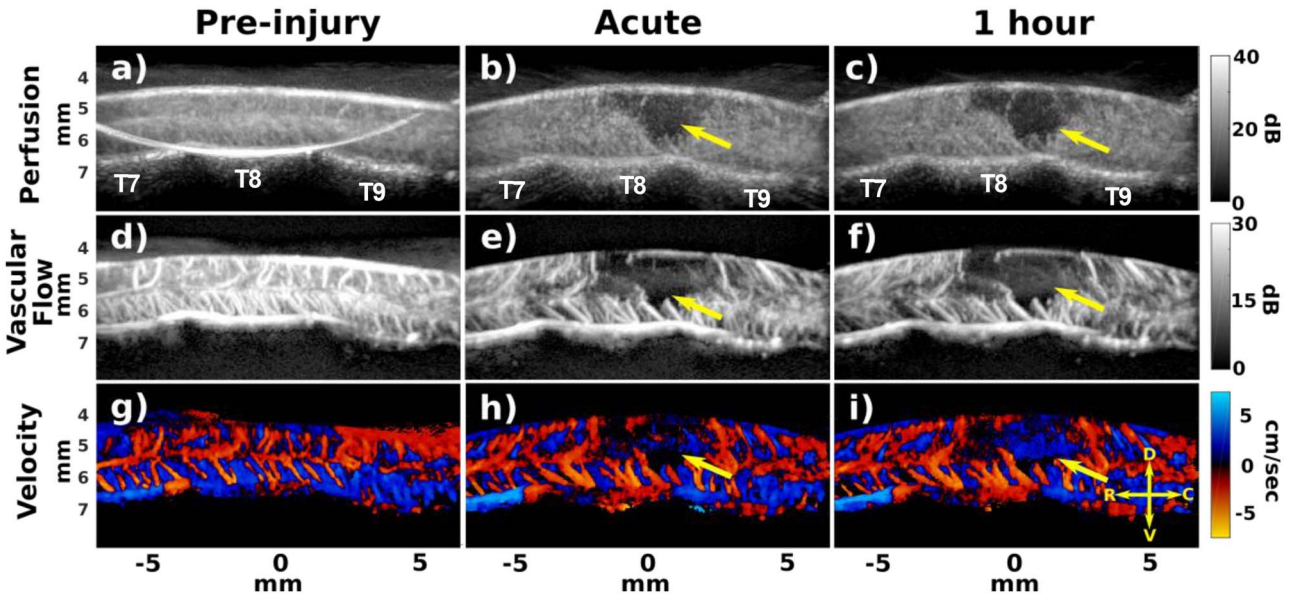

FIGURE 1. Ultrafast CEUS imaging can visualize hemodynamic changes in a rodent thoracic spinal cord model in vivo, in real time. Imaging was performed at preinjury (baseline, a, d, g), immediately after injury (~15 minutes postinjury, b, e, h), and 1 hour postinjury (c, f, i). Tissue perfusion (a–c), vessel flow (d–f), and flow velocity (g–i) images obtained at medial sagittal plane are depicted. Immediate and drastic loss of perfusion, vascular architecture, and reduce flow velocities (yellow arrows in b, c, e, f, h, i) were detected.

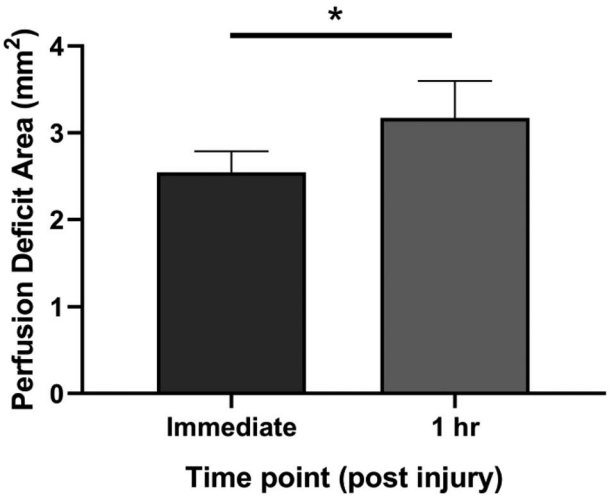

FIGURE 2. Longitudinal CEUS imaging detected area of perfusion deficit that increased with time after injury. Tissue perfusion images obtained from each animal ($n = 6$) were used to acquire area measurements of hypoperfusion. A large area of hypoperfusion developed immediately after injury (2.75 ± 0.26 mm^2) in all animals examined. At 1 hour postinjury, the area of hypoperfusion increased significantly (3.90 ± 0.27 mm^2; $^*p < 0.03$).

of patent vasculature within the injury zone. Moreover, CSAs in the ventral portion of the spinal cord showed clear distortions. In the intact spinal cord, CSAs extended into the central grey matter at regular angles (55–60°) with a cephalic inclination (Fig. 3). After SCI, ventral CSA rostral to the injury deflected in more rostral direction by $16.77 \pm 4.02°$, while CSA caudal to the injury was deflected toward the caudal direction by $21 \pm 1.5°$ compared to the intact spinal cord. These alterations in vessel architecture after contusion injury may affect blood supply to the penumbral zone of the injured spinal cord.

Using power Doppler processing, we obtained blood flow velocities in patent vessels within the spinal cord (Fig. 1g–i). We found that within the intact spinal cord, arterial and venous blood flows were at 5.04 ± 0.63 and 1.58 ± 0.47 cm/s, respectively (Fig. 4). After a contusion injury, there was a significant reduction in blood flow velocities in arterial vessels such that arterial flow velocities were 3.02 ± 0.23, 1.18 ± 0.16, and 3.26 ± 0.48 cm/s at rostral, epicenter, and caudal segments, respectively. After injury, venous flow was not detectable in the injury epicenter and there were also significant drops in venous flow velocities in segments rostral (1.4 ± 0.07) and caudal (0.8 ± 0.16 cm/s) to the injury.

DISCUSSION

Understanding acute hemodynamic alterations of the injured spinal cord may support development and implementation of novel neuroprotective strategies following SCI. Here, we describe the use of novel ultrasound acquisition and processing strategy to evaluate hemodynamic changes in vivo, in real time using a rodent SCI model.

Ultrasound imaging, especially CEUS imaging, has distinct advantages over other imaging techniques such as magnetic resonance imaging or perfusion computed tomography, for examining hemodynamic characteristics of the spinal cord. These include high spatial and temporal resolution, real-time imaging, cost effectiveness, lack of radiation, and its availability in operating room. Here, we report our novel

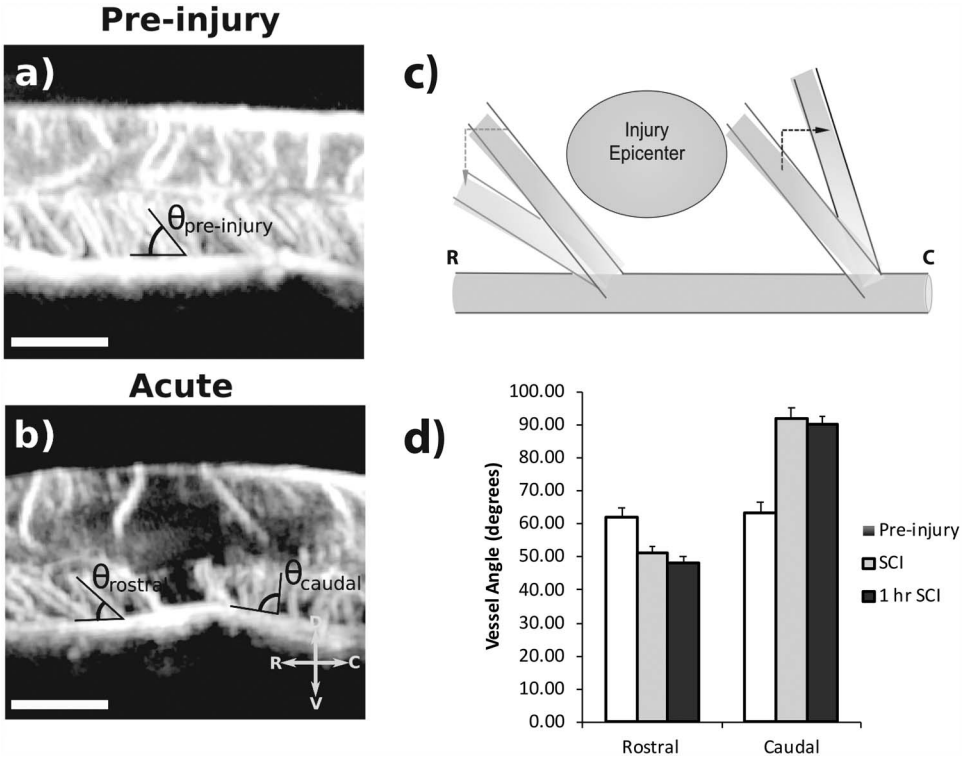

FIGURE 3. tSCI results in dramatic change in vessel architecture. Ventral intraspinal vessels inclined in the rostral direction (a). After tSCI, ventral CSAs rostral to the injury center inclined to a more rostral direction (b and c), thereby decreasing the angles, while ventral CSAs caudal to the injury center inclined to a more caudal direction and increased their angles (d). Scale bar = 1 mm.

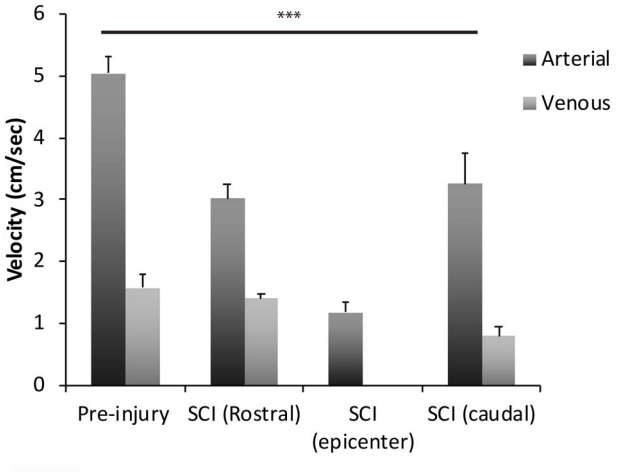

FIGURE 4. tSCI results in reduction in vessel flow velocities. Vessel flow velocity estimates were performed for both atrial and venous intraspinal vessels. There was reduction in both arterial and venous flow velocities after tSCI compared to control values ($p < 0.0001$). In addition, both arterial and venous flows were significantly slower in both rostral and caudal perilesional areas; ($p < 0.01$) and ($p < 0.02$), respectively.

CEUS Doppler imaging approach, which enables assessment of tissue perfusion and determination of structural and functional integrity of the spinal vasculature in the rodent spinal cord before and after a contusion SCI.

Sufficient blood flow or tissue perfusion is essential for proper oxygenation and the health of spinal tissue especially after injury. The SCI results in significant reduction in local blood flow and perfusion in the injury center with hypoperfusion in the penumbral areas over time.[6] Indeed after the primary injury, ischemia is thought to be a major contributor to the expansion of the injury during the secondary phase.[6,13] Current trauma guidelines for patients with acute SCI recommend maintenance of the mean arterial blood pressure at 85–90 mmHg for the first 7 days after injury, specifically attempting to limit local tissue hypoperfusion and thereby reducing secondary damage.[14] Surgical decompression of the contused spinal cord by aligning and stabilizing the spinal column and resection of impinging bone fragments within 24 hours after injury has been established as a widely accepted treatment option as it may partially restore local blood flow and improve functional outcomes.[15–19] However, a major limiting factor in evaluating clinical guidelines and experimental treatment strategies for increasing local blood flow has been the lack of a real-time assessment of local tissue perfusion and blood flow. In the current report, we show that CEUS can be used to monitor and evaluate local tissue perfusion after a contusion-type injury to the spinal cord in rodent in real time.

Previously, our group has shown that after a compression-type injury to the spinal cord, there was a loss of tissue perfusion in the injury epicenter.[7] Here, we replicate this finding in a more clinically relevant rodent SCI model, which uses a contusion-type injury instead of a compression-type injury. Similar to the compression-type injury, contusion SCI

also resulted in loss of perfusion and blood flow in an area of ~ 2.5 mm^2 immediately after injury. Interestingly, this original area of perfusion deficit increased with time after injury, from 2.5 to ~ 3.6 mm^2 (Fig. 2). This observation indicates that CEUS imaging has the temporal and spatial resolution needed to follow injury-induced progression of perfusion deficits over time. Our finding that the hypoperfused lesion site expands after injury confirms findings from Soubeyrand et al., where the authors used B-mode ultrasound images to estimate area of hemorrhage and found that it increased with time after contusion SCI in a rodent model.[20]

Injuries to the spinal cord can also result in dramatic disruption of vascular architecture in preclinical rodent models.[21,22] Cao et al. used synchrotron radiation microtomography (SRμCT) to examine altered micro- and macrovasculature in both 2D and 3D after injury to the spinal cord.[21] Similar to the findings detailed in the current article, Cao et al. showed dramatic loss of micro- and macrovessels in the injury epicenter. Moreover, alterations in vascular architectures (changes in ASA angle after injury) were also observed in both studies. Clinical investigations have corroborated several types of alteration of vessel architecture such as tortuosity, vessel angle changes, twisting, or kinking.[23] Clinically tortuous arterial vessels have been linked to ageing,[24] atherosclerosis,[25] and hypertension[26], and other vascular diseases and abnormalities. Tortuous vessels in the spine are also associated with ischemic damage to the surrounding tissue.[27] Severely tortuous arteries can also hinder the blood flow and lead to ischemic insults of distal organs.[28] Additional studies are needed to determine whether structural changes in vessel architecture/angel can be used to estimate the blood flow to the surrounding tissue. A major technical difference of note is that our technique, CEUS Doppler imaging, allowed real-time visualization of spinal vascular architecture in vivo, while the SRμCT method requires sacrifice of the animal. SRμCT method has better resolution in discerning smaller vessels ($\sim$10 μm), whereas our current CEUS Doppler method has a resolution of 100 μm. Nonetheless, the advantage of CEUS Doppler imaging for intraoperative visualization of intraspinal changes in vessel architecture acutely after SCI is apparent.

Alterations in blood flow velocities within vessels can predict abnormal blood supply to the tissue.[29–31] Although blood flow kinetics of peripheral vessels (eg, carotid artery) are well characterized, blood flow characteristics of intraspinal vessels are rarely studied due to technical limitations. Utilizing our novel CEUS Doppler imaging technique, we were able to estimate velocity of blood flow in both arterial and venous vessels (>100 μm). We characterized flow velocities in both arterial and venous vessels at preinjury and acutely after SCI and showed that there were velocity reductions in both types of vessels, with significant velocity reduction in arterial vessels. This finding is significant because acute reduction in vessel flow velocities maybe used to predict tissue dysfunction or tissue at risk for secondary damage after injury.

LIMITATIONS OF THE CURRENT STUDY AND FUTURE DIRECTION

We note several limitations of the current study. First, important hemodynamic changes after SCI are likely to progress well beyond 1-hour postinjury. Indeed, therefore, we are currently undertaking longitudinal studies detailing blood flow changes after SCI for up to 7 days postinjury. To further facilitate longitudinal studies, we have recently developed a transcutaneous CEUS imaging protocol using acoustic window created by the original surgery. That despite some degree of signal attenuation of the ultrasound signal in the subcutaneous tissue, transcutaneous CEUS allowed for reliable estimation of local hypoperfusion. In the current study, CEUS imaging from a single mid-sagittal plane showed significant alterations to tissue perfusion, vessel architecture, and flow velocities after SCI. Contusion-type injury to the spinal cord will likely result in the development hemodynamic changes in 3D.[18] Therefore, we are currently developing innovative methods to obtain 3D ultrafast CEUS imaging for future studies. Ultrasound contrast agents can also be used to bind to specific biologically relevant targets, hence opening up future opportunities for molecular imaging studies that may be important in tracking injury progression and recovery after SCI.

Ultrasound-based blood flow measurements are well suited to be translated into clinical use. First, ultrasound devices are readily available in the operating rooms, and hence surgeons and technical staff are already familiar with their use. Moreover, contrast agents are currently Federal Drug Administration approved for cardiac indications, thus allowing for off-label use for clinical trials. Currently, our laboratory is developing strategies to translate CEUS imaging of rodent perfusion measurements to be applied to patients with acute SCI. Additionally, the development of noninvasive transcutaneous real-time intravital imaging of CEUS will also provide additional accessibility to use this technology to evaluate and monitor local blood flow change after injury and treatment.

CONCLUSION

Results obtained in the current study demonstrate that ultrafast CEUS imaging provides the temporal and spatial resolution needed to detect and monitor acute hemodynamic in a rodent tSCI model. Future work will attempt to translate this technology for clinical application aiming to guide and monitor therapeutic interventions in patients with acute SCI.

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