Basic Neuroscience

A perfusion procedure for imaging of the mouse cerebral vasculature by X-ray micro-CT

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HIGHLIGHTS

- Brain samples perfused with contrast agent have high inconsistency in the filling of the posterior cerebral circulation.
- We revised the Microfil perfusion protocol, in order to reduce the variability of the outcome in samples.
- The Microfil is first perfused through the posterior circulation by blocking the flow to the anterior circulation.
- A workflow is provided to verify the successful completion of each surgical step.
- The cerebellum shows 6.9%, and the midbrain about 8.7% increase in the percentage of vessel segments.

ARTICLE INFO

ABSTRACT

Background: Micro-CT is a novel X-ray imaging modality which can provide 3D high resolution images of the vascular network filled with contrast agent. The cerebrovascular system is a complex anatomical structure that can be imaged with contrast enhanced micro-CT. However, the morphology of the cerebrovasculature and many circulatory anastomosis in the brain result in high variations in the extent of contrast agent filling in the blood vessels and as a result, the vasculature of different subjects appear differently in the acquired images. Specifically, the posterior circulation is not consistently perfused with the contrast agent in many brain specimens and thus, many major vessels that perfuse blood to the midbrain and hindbrain are not visible in the micro-CT images acquired from these samples.
New method: In this paper, we present a modified surgical procedure of cerebral vasculature perfusion through the left ventricle with Microfil contrast agent, in order to achieve a more uniform perfusion of blood vessels throughout the brain and as a result, more consistent images of the cerebrovasculature. Our method consists of filling the posterior cerebral circulation with contrast agent, followed by the perfusion of the whole cerebrovasculature.
Results: Our histological results show that over 90% of the vessels in the entire brain, including the cerebellum, were filled with contrast agent.
Comparison with existing method: Our results show that the new technique of sample perfusion decreases the variability of the posterior circulation in the cerebellum in micro-CT images by 6.9%.
Conclusions: This new technique of sample preparation improves the quality of cerebrovascular images.

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1. Introduction

The imaging of blood vessels is a common method to acquire information about the development of vascular systems and their connectivity, the blood perfusion into different organs, and the pathology of vessels (van den Wijngaard et al., 2013; Young et al., 1979; Pathak et al., 2008). Ultrasound, magnetic resonance angiography (MRA) and X-ray angiography are common methods to image vessels and are useful for calculating the blood perfusion and finding pathologies in the vessel walls. In recent years, micro-CT imaging has been utilized to image small animals such as mice for vascular biology research (Jorgensen et al., 1998; Holdsworth and

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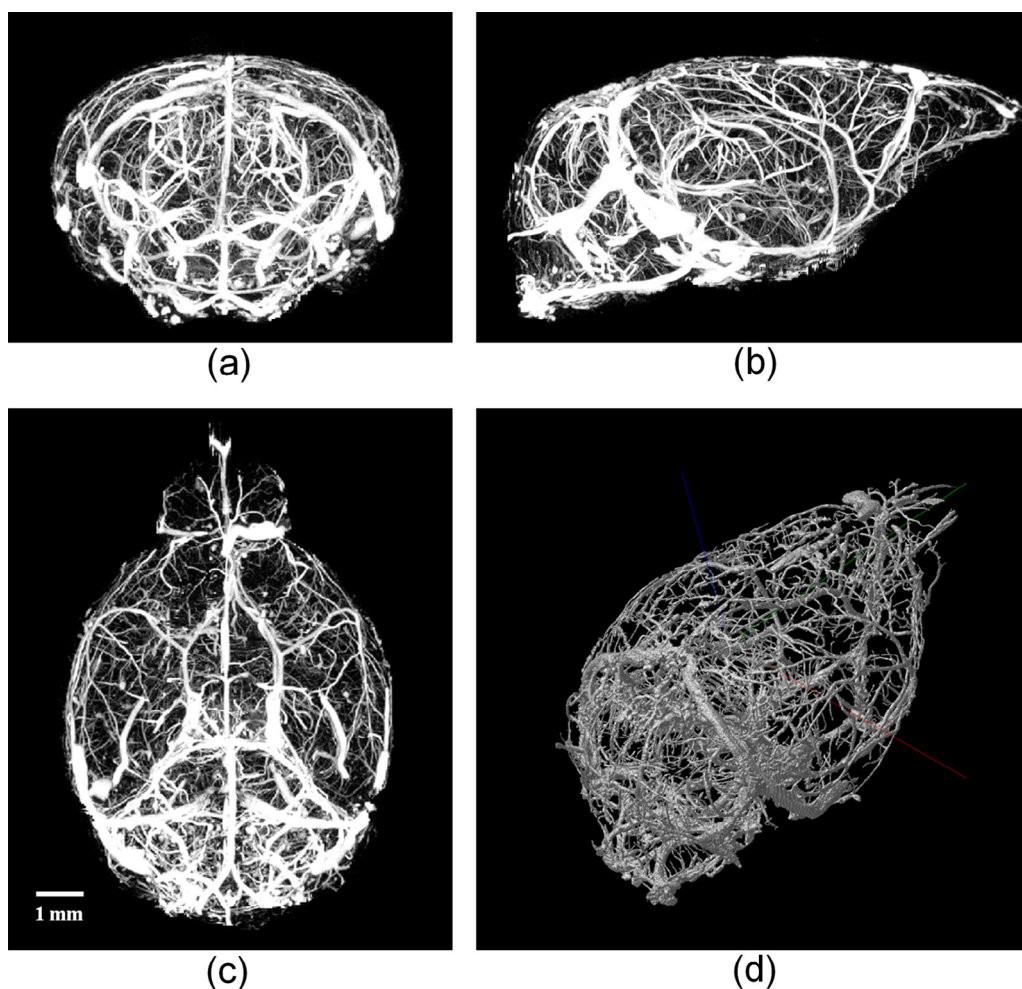
Fig. 1. Maximum intensity projections (MIP) and 3D rendering of the micro-CT image of a wild-type C57BL/6 mouse brain which was perfused with Microfil using our new method of contrast agent perfusion and was imaged with 20 μm isotropic resolution. (a) Coronal, (b) sagittal, and (c) axial MIP representation, and (d) the 3D rendering.

Thornton, 2002). Due to its high resolution and short scan time, micro-CT is a suitable imaging method for imaging microvasculature of intact rodent organs. The use of low viscous CT contrast agents that fill the lumen of vessels has made it possible to acquire high-resolution 3D images of vascular systems such as kidney and placenta (Bentley et al., 2002; Nordsletten et al., 2006; Marxen et al., 2006; Rennie et al., 2007; Yang et al., 2010). The cerebrovascular casting in mouse using casting agents can be used to image the brain sample with bright field microscopy, stereomicroscopy, scanning electron microscopy, as well as micro-CT (Walker et al., 2011; Cruise et al., 2009; Krucker et al., 2006; Heinzer et al., 2006). Specifically, Microfil contrast agent, a low viscous silicone rubber injection compound containing lead pigments, has been used to perfuse the vascular system to be imaged *ex vivo* with micro-CT (van den Wijngaard et al., 2013; Chugh et al., 2009; Rennie et al., 2010; Chutkow et al., 2002; Daneyemez, 1999; Vasquez et al., 2011; Marxen et al., 2004). In this perfusion method the lumen of vessels are filled with contrast agent. The lead pigments in the Microfil provide high contrast compared to the background tissue needed to acquire a complete high resolution 3D image of the vascular structure. The perfusion technique presented here is relatively easy to perform and can be used to visualize the whole vascular system. In addition, the perfused brain samples can be embedded in paraffin and be used for histological sectioning in order to examine the cells and tissues, for example to stain and visualize the endothelial cells in the vessel walls. As an example of value of this technique, Fig. 1 shows the sagittal, coronal, and axial maximum intensity

projections (MIPs) representation of a wild-type C57BL/6 mouse brain perfused with contrast agent and imaged with 20 μm isotropic resolution. The arteries, veins and sinuses in mouse brain can be identified on the 3D high resolution micro-CT image.

A retrospective analysis has been performed on previous studies from our group. This analysis showed that despite the high resolution 3D images that can be acquired from contrast agent perfused cerebral arteries and veins and the high density of the visible cerebral vessels in the micro-CT images, the connectivity and branches of cerebrovascular structure showed high variations, especially in the cerebellum area. We hypothesize that the variations and artifacts introduced by the perfusion procedure can be the cause for differences among images of different subjects. Fig. 2 shows the sagittal view MIP representation of micro-CT images taken from two wild-type C57BL/6 mice. A MIP representation of a 2 mm slab centred at the mid-sagittal plane for the samples are also shown in the right column of Fig. 2. As it can be seen from this figure, some major vessels, such as arteries perfusing the cerebellum, are completely or partially missing from the micro-CT image shown in the second row. This difference in the images of the two samples is not likely to be caused by underlying physiological differences. The two samples share similar genetic background and environment. Also, missing major arteries in the brain, as can be seen in the second row of Fig. 2, would likely have led to premature death or a severe behavioural deficit. Since the imaging protocol is also consistent among all the samples, the likelihood is low of the variations originating from the instrumental sources.
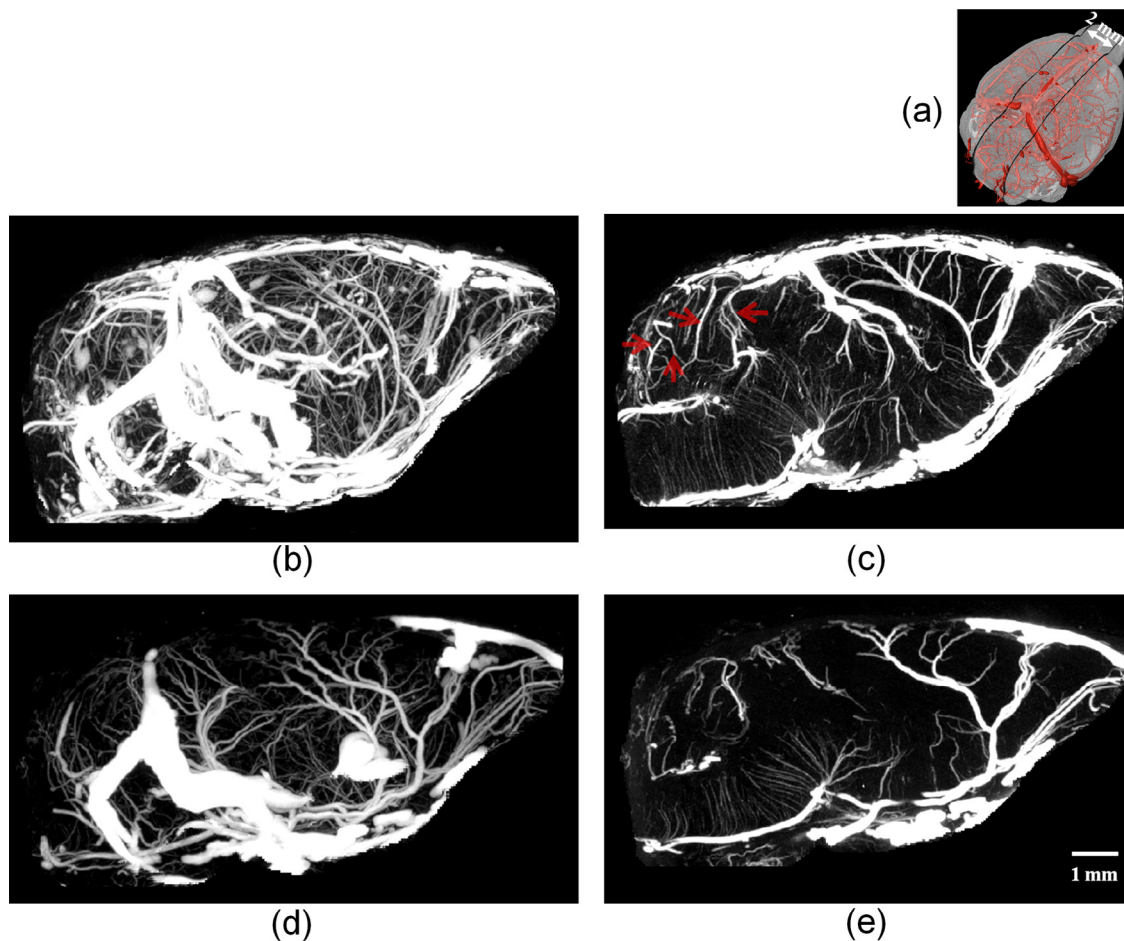
Fig. 2. The sagittal view of micro-CT image MIP from two wild-type C57BL/6 mice perfused with the original perfusion method, in which the whole cerebrovasculature is perfused simultaneously. (a) A presentation of the selected 2 mm slab centred at the mid-sagittal plane. (b) Sagittal view MIP of a well perfused sample, (c) sagittal view MIP of a 2 mm slab centred at the mid-sagittal plane of the same mouse, (d) sagittal view MIP of the poor perfusion sample, and (e) sagittal view MIP of a 2 mm slab centred at the mid-sagittal plane of the same mouse. Comparison of the images from these two samples shows high variation in the number and extent of vessels in the brain, especially in the midbrain and hindbrain. The arrows highlight cerebellar areas in which some vessels are present in the top image and are missing from the bottom image.

1.1. Perfusion inconsistency hypothesis

Chugh et al. (2009) have tested the perfusion variability of five specimens in four brain locations: frontal cortex, striatum, hippocampus and superior colliculus. The specimens were paraffin embedded and coronal sections of 5 μm thickness were cut. These sections were stained with hematoxylin and the percentage of lumens filled with Microfil were calculated under 20× magnification. Their results showed no significant difference between the perfusion percentage in different locations and their calculations showed that 93±3% of the vessels were perfused by Microfil. Our retrospective study of micro-CT images were consistent with the histology findings reported by Chugh et al. (2009) in the cerebral cortex, which is perfused by the anterior circulation including internal carotid artery (ICA) and its branches. However, in their study, no section was cut from the cerebellum and the extent of the contrast agent filling in the cerebellar vessels was not reported. Our study of the micro-CT images of brain samples perfused with Microfil contrast agent showed that the contrast agent perfusion consistency, which is seen through the cerebral cortex, is not present in the cerebellum. The cerebellum is mainly perfused by the posterior circulation including vertebral and basilar arteries and their branches. Comparison of micro-CT images of different brain samples in the cerebellum as depicted in Fig. 2 demonstrates a high variability of the perfused vessels with contrast agent.

The cerebral vascular system consists of anterior and posterior circulations. The anterior circulation consists of two internal carotid arteries (ICA), which first gives two branches of the posterior cerebral arteries (PCA) and then, bifurcates into middle cerebral artery (MCA) and anterior cerebral artery (ACA) (Scremin and Holschneider, 2011). These vessels perfuse the cerebral cortex and circulate about 75% of the total cerebral blood volume (CBV) (Scheel et al., 2000). The posterior circulation consists of two vertebral arteries that join into the basilar artery and branches into superior cerebellar artery (SCA) (Scremin and Holschneider, 2011). The posterior circulation perfuse about 25% of the CBV to the midbrain and hindbrain including the cerebellum (Scheel et al., 2000). The two distinct circulations are usually connected by unilateral or bilateral small connecting vessels called posterior communicating arteries, which forms a collateral circulation path called the circle of Willis. The mean diameter of the ICA as measured by Kidoguchi et al. is 162.6±10.87 μm (Kidoguchi et al., 2006) and the mean diameter of basilar artery is about 132±4 μm (Faraci et al., 2006). Fig. 3 shows the ventral view of a brain sample perfused with Microfil with the skull removed. The Microfil appears as the yellow color filling and the major arteries constructing the circle of Willis are identified on the sample.

As explained, the blood vessels in the posterior circulation are smaller in diameter and thus, have higher resistance. Some mouse strains, such as C57BL/6, mostly lack both posterior communicating arteries or have very weak connections and thus, have
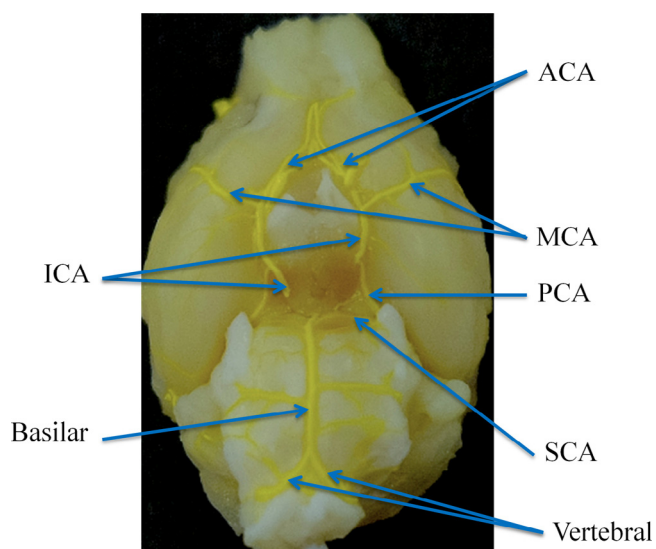
Fig. 3. The ventral view of a C57BL/6 mouse brain perfused with Microfil. The cerebral arteries constructing the circle of Willis are labelled. Anterior circulation consists of internal carotid artery (ICA), posterior cerebral artery (PCA), middle cerebral artery (MCA), and anterior cerebral artery (ACA). Posterior circulation consists of vertebral arteries and the basilar artery bifurcating into superior cerebellar arteries (SCA).

an incomplete circle of Willis (Beckmann, 2000; Wellons et al., 2000). In addition, Microfil has a higher viscosity compared to blood (Marxen et al., 2004; Vasquez et al., 2011). Our hypothesis for the high variations in cerebellum vascular contrast agent filling is that the aforementioned factors cause the Microfil to be mostly perfused through the ICAs and not through the vertebral arteries. In specimens missing the posterior communicating arteries, the inconsistency in contrast agent filling of the posterior circulation is more severe.

In this paper, we revise the cerebrovascular Microfil perfusion protocol through the left ventricle of the heart described in Chugh et al. (2009), in order to reduce the variability of the outcome in samples and to make a meaningful comparison of the actual anatomical variations among cerebral vasculature of subjects, using the high resolution micro-CT imaging, possible. In the following sections, we detail the Microfil perfusion protocol and the revised version of the method. We will also provide qualitative and quantitative results comparing the old and new methods of Microfil perfusion and the acquired micro-CT images.

2. Materials and methods

2.1. Procedure overview

Microfil (Flow TEch, Inc., Carver, MA, USA) is a radio opaque silicone rubber containing lead chromate and lead sulfate particles. An important property of Microfil that makes it suitable for *ex vivo* vascular imaging is its low shrinkage (Cortell, 1969). The method of filling the cerebral vasculature with Microfil has been detailed in Chugh et al. (2009). The mice are anesthetized with intraperitoneal injection (IP) of 100 μg ketamine (Pfizer, Kirkland, QC, Canada), 20 μg xylazine (Bayer Inc., Toronto, Canada) and 3 μg acepromazine maleate (Vetoquinol, Lavaltrie, QC, Canada) per gram of body weight. Acepromazine maleate is a vasodilator that keeps the arteries at the diameter of rest (Arras et al., 2001). The animal then receives an IP injection of heparin (200 U) (Organon Canada Ltd., Toronto, Canada) which is used as anticoagulant to prevent the blood from clotting in the vessels after the animal's death. The surgery consist of first clamping the vena cava and the descending

aorta. A 24-gauge IV catheter (Becton Dickinson Infusion Therapy System Inc., UT, USA) is then inserted into the left ventricle of the heart and is sealed in place using adhesive Locatite 404 (McMaster-Carr, GA, USA). The IV catheter is connected to a pressure-controlled pump (Model PS/200, Living Systems Instrumentation, VT, USA).

The blood in the cerebral vessels is drained out through an incision on the right atrium and is replaced by warm heparinized (5 U/mL) phosphate buffered saline (Wisent Inc., St-Bruno, QC, Canada) which is perfused into the vascular system at 50 mmHg for 5 min at a filling rate of 2 mL/min. The Microfil is perfused right after the complete drainage of blood. Microfil is perfused at room temperature at 150 mmHg for 10 min at filling rate of 0.25 mL/min. Then the pump is stopped and the slit on the right atrium is sealed. The pump is set to 30 mmHg, the normal capillary pressure (Burton, 1972; Li, 2004) and the Microfil polymerizes in about 90 min.

2.2. Revised procedure

We propose a revision to the perfusion protocol detailed above for filling the mouse cerebrovasculature with contrast agent, which addresses the inconsistent perfusion of the vessels in the posterior circulation. In the revised protocol, the Microfil is first perfused through the posterior circulation by blocking the flow to the anterior circulation.

2.2.1. Reagents

- Microfil (Flow TEch, Inc., Carver, MA, USA)
- Ketamine (Pfizer, Kirkland, QC, Canada)
- Xylazine (Bayer Inc., Toronto, Canada)
- Acepromazine maleate (Vetoquinol, Lavaltrie, QC, Canada)
- Heparin (200 U) (Organon Canada Ltd., Toronto, Canada)
- Phosphate buffered saline (Wisent Inc., St-Bruno, QC, Canada)
- Formalin phosphate (Fisher Scientific Company, Ottawa, Canada)
- Formic acid (Fisher Scientific Company, Ottawa, Canada)
- Adhesive Locatite 404 (McMaster-Carr, GA, USA)

2.2.2. Equipments

- Pressure-controlled pump (Model PS/200, Living Systems Instrumentation, VT, USA)
- 23-gauge butterfly needle (Becton Dickinson VACUTAINER Systems, NJ, USA)
- SkyScan 1172 high-resolution micro-CT scanner (Bruker micro-CT, Belgium)

2.2.3. System preparation • Timing ~10 min
1. Set up the pressure-controlled pump by connecting the required tubing and 2 empty syringes, one for the saline buffer and one for the Microfil contrast agent.
2. Warm up the heparinized (5 U/mL) phosphate buffered saline which will replace the blood in the vasculature.
3. Fill all tubing with the buffer in order to remove air bubbles from the tubes of the pressure pump.

2.2.4. Animal handling • Timing ~15 min
4. Inject 100 μg ketamine, 20 μg xylazine, and 3 μg acepromazine maleate per gram of body weight into mice intraperitoneally.
    CAUTION - Verify animal's anesthetic state by pinching toes. The mice under full anesthesia do not demonstrate muscular reaction.
5. Inject 200 U of anticoagulant agent, heparin intraperitoneally.
6. Remove the chest hair using hair removal cream.

2.2.5. Surgical procedure • Timing ~2 h

7. Cut through the chest and neck of the animal and expose the heart, lungs, liver and trachea.
8. Clamp the vena cava and the descending aorta.
9. Insert the 23-gauge butterfly needle into the left ventricle. Fix it using adhesive Locatite 404, and connect to the pressure-controlled pump.
10. Cut a slit on the right atrium, set the pressure-controlled pump to 50 mmHg for 5 min to replace the blood with the warm heparinized phosphate buffered saline.
11. Stop the pump.
12. Clamp the two common carotid arteries, which run parallel to the trachea.
13. Mix the Microfil solution in the syringe connected to the pressure-controlled pump and switch the pump to run the Microfil solution. Set the pressure to 150 mmHg for about 2 min.
     CAUTION - It takes about 2 min for the Microfil to circulate through the posterior cerebellar vascular system and to be drained from the slit on the right atrium. This means that the Microfil has circulated through the brain and has flowed back to the heart.
14. While the pump is running, unclamp the common carotid arteries and continue the perfusion at 150 mmHg for 20 min throughout the entire cerebral circulation.
15. Seal the slit on the right atrium and set the pump to 30 mmHg for 90 min until the Microfil polymerization is completed.

2.2.6. Sample preparation for imaging • Timing ~3 days

16. Dissect the skulls and remove the external tissue and lower jaw.
17. Fix the brain samples for 12 h at $4 \pm 1 \,^{\circ}$C in 10% buffered formalin phosphate.
18. Decalcify the skulls in 8% formic acid at room temperature for 48 h.
19. Place the samples in 1% agar gel.

2.3. Troubleshooting

The surgery for the cerebrovascular perfusion consists of many steps, each of which can affect the outcome of the perfusion. As a result, it is important to know the indicators that show successful completion of each step of the surgery. Fig. 4 shows the step-by-step checklist for monitoring the performance of the perfusion surgery. Here, we explain these indicators that should be checked for during the surgery.

The blocking of the vena cava and the descending aorta is important to prevent the heparinized phosphate buffered saline and the Microfil from circulating in the lower abdomen and limbs, reduce the total perfusion time and required Microfil volume. This is important because the polymerization of the Microfil begins in 20 min and as a result, the total time to circulate the Microfil through the region of interest should be as short as possible. In order to check that the clamping has been successful, liver should be monitored during the blood drainage stage. If the vena cava and the descending aorta are clamped successfully, the blood in the liver will not be drained out and the liver will remain its dark red color. To have a successful perfusion, the blood needs to be completely drained from the vessels first. To check for this, the tongue can be observed. The complete blood drainage should leave the vessels on the bottom of the tongue pale.

The insertion of the needle in the left ventricle of the heart is a delicate procedure. It is important to keep the needle straight and not to cut through into the right ventricle. If the needle is inserted into the right ventricle, the lung will be perfused with Microfil quickly and will turn yellow. This means that we will lose a large portion of the total Microfil volume and the pump needs to be run for longer to have the brain perfused with enough volume of Microfil. The correct insertion of the needle can be verified at the stage of blood drainage by verifying that the lung does not become pale and swollen.

The facial vessels and the vessels in the tongue branch off the common carotid artery. Observation of the tongue is a good indicator of successful clamping and unclamping of common carotid arteries. If the common carotid artery is clamped, the vessels on the bottom of the tongue should not be perfused by Microfil. Unclamping of right common carotid artery should be followed by perfusion of the right side of the tongue. The same applies to the left common carotid artery.

3. Results

In order to evaluate the revised perfusion protocol, 8 female C57BL/6 adult mice of age 16–20 weeks old were perfused with Microfil using the protocol detailed above and were CT scanned (SkyScan 1172 high-resolution micro-CT, Bruker micro-CT, Belgium). The images were reconstructed with isotropic cubic voxel size of 20 μm. The brain samples were embedded in paraffin and coronal sections of 5 μm thickness were cut from 5 different brain regions, including the frontal cortex, striatum, hippocampus, superior colliculus, and cerebellum. The sections were stained with hematoxylin and were compared with the histological sections acquired from brain samples prepared with the old perfusion protocol, under $40\times$ magnification using bright field microscope.

The images acquired from the brain samples perfused by the revised perfusion method demonstrated that all the major arteries, veins and sinuses, that are identified in mouse cerebrovascular atlases such as those presented in (Dorr et al., 2008; Scremin and Holschneider, 2011), are perfused successfully (Fig. 5). Comparison of the images from different samples showed higher consistency in the outcome of the contrast agent perfusion of the brain.

The histological sections from different regions of the brain samples prepared using the revised perfusion protocol were compared. The percentage of vessel lumens in the cerebellum that were filled with contrast agent were consistent with the percentage of vessels filled with contrast agent in the other four brain regions, with an average of >90% of vessels perfused. Our results were also consistent with the results reported in Chugh et al. (2009). The comparison of histological sections from the cerebellum of the brain samples prepared using the existing and revised perfusion protocols, as seen in Fig. 6, showed an increase in the number of cerebellar vessels filled with contrast agent using our revised perfusion protocol.

In order to compare the outcome of the existing brain perfusion protocol with our new revised method, we calculated the number of vessel branches in different regions of the brain including the cerebral cortex, cerebellum, midbrain, hippocampus and striatum in the 3D images acquired from the samples prepared by the two perfusion methods. Each micro-CT image was registered to an MRI anatomical atlas of the mouse brain, for the purpose of mapping the anatomical regions onto the micro-CT images (Dorr et al., 2008). The registration was done using manually selected landmarks as explained in Chugh et al. (2009). The mean value, maximum and minimum percentage of the number of vessel segments, which are defined as each vessel between two branching points in the vascular system, in the chosen anatomical regions with respect to the total number of vessel segments in the entire brain are shown for the micro-CT images acquired from samples perfused with the original and new Microfil perfusion methods in Table 1 left columns and right columns, respectively. ANOVA test was performed to determine the significantly different mean values reported for the two methods. The Levene's test was used to
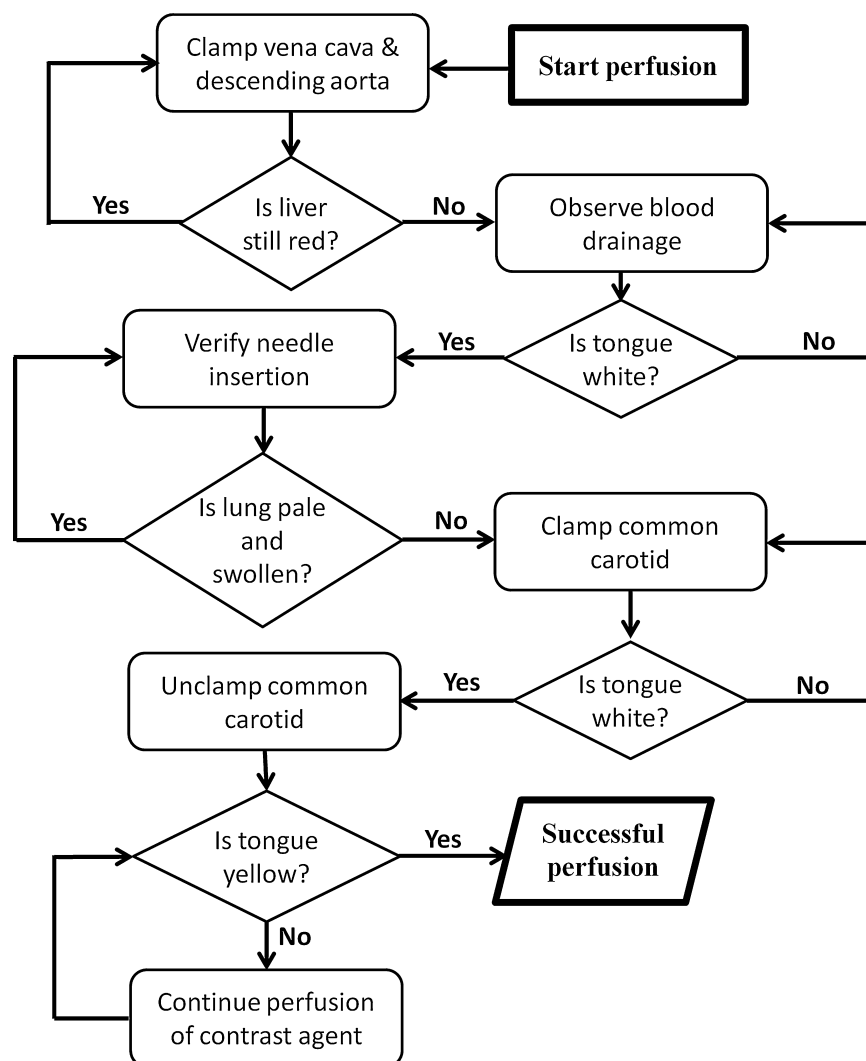
Fig. 4. The step-by-step checklist for monitoring the success of the perfusion surgery.

determine the significant difference between the two methods variances reported in the Table 1. The *p*-value for these two statistical test were reported as well.

In our experiments, we found that the successful completion of each perfusion step is critical in the overall success of the contrast agent filling of the entire cerebrovasculature. Thus, it is important to keep track of the surgical steps that are completed successfully and the steps that have gone wrong. All the samples need to be quality checked for these deficiencies. In a comparative study of the cerebrovasculature, the samples that were not perfused successfully need to be discarded, in order to prevent the perfusion deficits

from affecting the results of the study. In the present study, out of 15 C57BL/6 brain samples that were perfused with the new perfusion protocol, 3 were discarded due to a problem in the surgical procedure, such as the insertion of the needle into the right ventricle, which causes the superficial pulmonary vessels to be filled with Microfil.

4. Discussion

Comparing the results presented in Table 1 from the old and new perfusion protocols, shows that the percentage of vessel

Table 1
Comparison of the number of vessel segments in different neuroanatomical regions with respect to the total number of vessel segments in the entire brain, in 30 C57BL/6 wild-type mice perfused with Microfil using the old protocol (left column), and in 12 C57BL/6 wild-type mice perfused with the new protocol of Microfil perfusion (right column), imaged with 20 μm isotropic micro-CT.
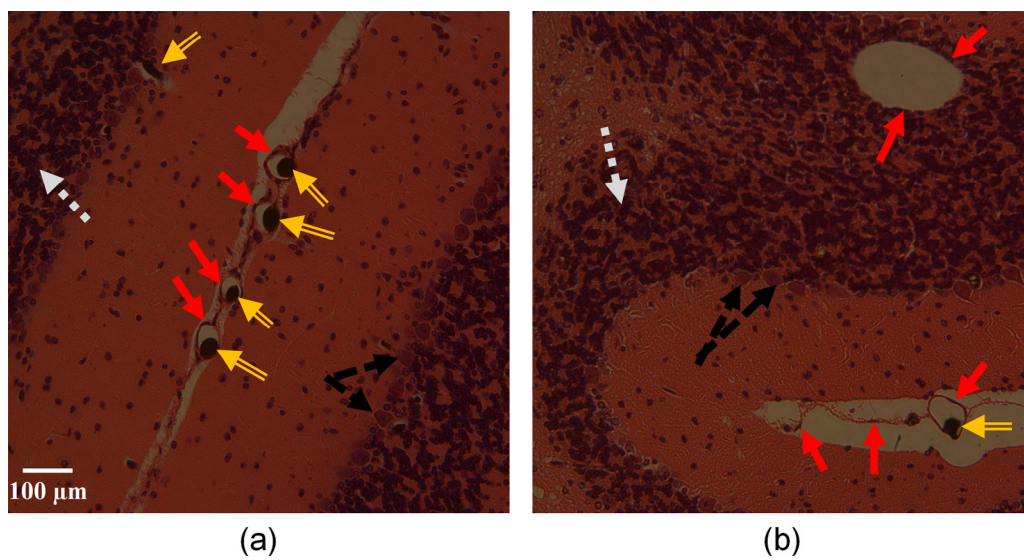
	Existing perfusion method			Proposed perfusion method			Mean comparison (ANOVA *p*-value)	SD comparison (Levene's *p*-value)
	Mean±SD(%)	Max	Min	Mean±SD(%)	Max	Min		
Cerebral cortex	31.91 ± 6.31	49.99	13.15	28.3 ± 5.21	37.15	20.66	0.15	0.97
Cerebellum	17.61 ± 4.88	27.73	4.78	24.53 ± 6.52	34.51	17.94	0.0021	0.19
Midbrain	16.79 ± 5.46	39.54	11.77	25.5 ± 3.71	30.89	18.68	0.00016	0.63
Hippocampus	7.46 ± 1.93	11.44	3.8	5.35 ± 0.95	6.05	3.16	0.0055	0.048
Thalamus and hypothalamus	7.16 ± 3.01	15.25	1.97	4.67 ± 1.2	5.76	2.01	0.029	0.014
Olfactory bulbs	17.56 ± 9.19	42.78	8.67	9.4 ± 3.74	14.11	1.44	0.00017	0.397
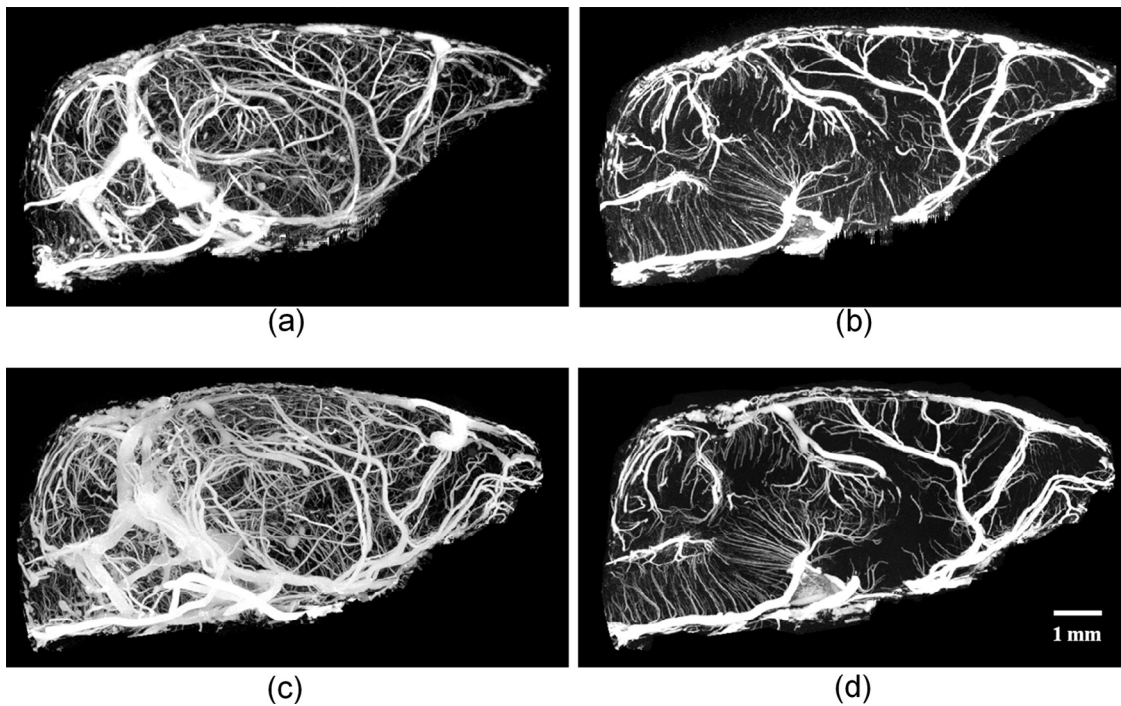
Fig. 5. The sagittal view of micro-CT image MIP from two wild-type C57BL/6 mice perfused with the new version of the perfusion method. (a) The sagittal view MIP and (b) the sagittal view MIP of a 2 mm slab centred at the mid-sagittal plane of a perfused sample. (c) The sagittal view MIP and (d) the 2 mm mid-sagittal slab MIP of another perfused sample. Comparison of the images from these two samples shows consistent perfusion in both samples as well as throughout the brain. The number and extent of vessels in the brain, especially in the midbrain and hindbrain are comparable, as seen in the images in the right column.

numbers are increased significantly in midbrain and hindbrain ($p < 0.00016$ for midbrain and $p < 0.0021$ for cerebellum). Specifically, the cerebellum shows 6.9%, and the midbrain about 8.7% increase in the percentage of vessel segments. The percentage of vessel segments in the cerebral cortex is not affected considerably, as expected ($p < 0.15$). The cerebral cortex contains the highest percentage of vessel segments among the brain regions and is perfused by the anterior circulation system. The high percentage of vessel

segments in the cortex demonstrate that the contrast agent filling through the anterior circulation is not affected negatively by the initial clamping of the common carotid arteries and the vessels are filled with the contrast agent through the ICAs after we unclamp the common carotid arteries. On the other hand, the standard deviations reported in Table 1 show decrease in all brain regions studied, except the cerebellum. The decrease in the standard deviation is significant in the hippocampus, thalamus and hypothalamus

Fig. 6. Histological staining of the cerebellum with hematoxylin under 40× magnification. (a) A C57BL/6 brain sample perfused using the revised protocol, and (b) a C57BL/6 brain sample prepared using the existing perfusion method. The white dotted arrows show the granule cells and the black dashed arrows show the Purkinje cells in the cerebellum layers. The red solid arrows indicate the flat thin endothelial cell nuclei around the vessel lumen. Microfil appears as a dark blob inside the lumen, indicated by yellow double-lined arrows. The vessel lumens in the left image are filled with Microfil using our revised protocol. The empty lumens in the image on the right show that many cerebellar vessels are not filled with contrast agent using the existing perfusion protocol.

($p < 0.048$ and $p < 0.014$, respectively). These results confirm that the revised perfusion method can improve the perfusion result, especially in the midbrain and hindbrain.

The study of the histological sections from the brain specimens perfused using the new protocol confirms the results published by Chugh et al. (2009) for the coronal sections acquired from frontal cortex, striatum, hippocampus, and superior colliculus. However, no result was previously reported for the histological sections from the cerebellum to assess the percentage of vessels perfused with Microfil. Our histology study confirms that in the new protocol the percentage of perfused vessels in the sections from the cerebellum are consistent with the rest of the brain.

5. Conclusion

We have proposed a surgical procedure for perfusion of the mouse brain vascular system with Microfil, which addresses the incomplete perfusion of the posterior circulation. The complete and consistent perfusion of the brain vasculature improves the image quality of the cerebrovascular system using micro-CT. The accuracy of the studies using this method of vascular imaging is increased and the anatomical and morphological differences caused by the underlying biology in the acquired images are highlighted due to the reduction of the variations caused by the imaging protocol.

Acknowledgements

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