In vivo imaging in experimental spinal cord injury – Techniques and trends

Vanessa Hubertus a, Lea Meyer a, Laurens Rooils b, Lilly Waldmann a, Melina Nieminen-Kelha a, Michael G. Fehlings b,1, Peter Vajkoczy a,*,1

a Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität zu Berlin, And Berlin Institute of Health, Department of Neurosurgery, Berlin, Germany
b Toronto Western Hospital, Department of Neurosurgery and Spinal Program, University Health Network, Toronto, Canada

A R T I C L E   I N F O

Keywords:
- Spinal cord injury
- Spinal cord regeneration
- In vivo studies
- Animal studies
- Modern imaging
- In vivo imaging

A B S T R A C T

Introduction: Traumatic Spinal Cord Injury (SCI) is one of the leading causes of disability in the world. Treatment is limited to supportive care and no curative therapy exists. Experimental research to understand the complex pathophysiology and potential mediators of spinal cord regeneration is essential to develop innovative translational therapies. A multitude of experimental imaging methods to monitor spinal cord regeneration in vivo have developed over the last years. However, little literature exists to deal with advanced imaging methods specifically available in SCI research.

Research Question: This systematic literature review examines the current standards in experimental imaging in SCI allowing for in vivo imaging of spinal cord regeneration on a neuronal, vascular, and cellular basis.

Material and Methods: Articles were included meeting the following criteria: experimental research, original studies, rodent subjects, and intravital imaging. Reviewed in detail are microstructural and functional Magnetic Resonance Imaging, Micro-Computed Tomography, Laser Speckle Imaging, Very High Resolution Ultrasound, and in vivo microscopy techniques.

Results: Following the PRISMA guidelines for systematic reviews, 689 articles were identified for review, of which 492 were sorted out after screening and an additional 104 after detailed review. For qualitative synthesis 93 articles were included in this publication.

Discussion and Conclusion: With this study we give an up-to-date overview about modern experimental imaging techniques with the potential to advance the knowledge on spinal cord regeneration following SCI. A thorough knowledge of the strengths and limitations of the reviewed techniques will help to optimally exploit our current experimental armamentarium in the field.

1. Introduction

Traumatic spinal cord injury (SCI) is one of the world’s leading causes of disability (Singh et al., 2014; Cripps et al., 2010; Majdan et al., 2017; Badhiwala et al., 2018). No curative therapy exists, and treatment is limited to supportive care. In the past decades, experimental research brought tremendous progress to the basic knowledge of the complex pathophysiology underlying SCI, as well as to the endogenous regenerative responses of the spinal cord. With advancing knowledge, research is shifting from the general characterization of SCI pathophysiology to the potential amelioration of spinal cord regeneration via targeting endogenous repair mechanisms (Monje, 2021). Experimental studies using longitudinal in vivo imaging play a significant role in the characterization of potential targets for advancing spinal cord regeneration.

Experimental imaging of CNS pathologies has evolved widely over the past decades. In comparison to classical histological tissue analysis, longitudinal in vivo imaging allows for an increasingly accurate morphological and functional tissue analysis in the same subject at different time points. With high dynamics and a high spatial resolution, longitudinal in vivo imaging can be performed non-invasively and therefore repeatedly. This does not only increase comparability, but also reduces the necessary group sizes in animal experimental research. In SCI, in vivo imaging evolved over the last years from the mere morphological assessment of trauma size and regeneration to the possibility of live tracking of spinal cord injury and regeneration on a neuronal, vascular, and even on a cellular level. These techniques allow...
furthermore for real-time tracking and longitudinal follow-up of the regenerative capacities of experimental therapies.

However, little literature exists to deal with the multitude of advanced experimental imaging methods specifically available in SCI research, and their capacities to enhance the understanding of spinal cord regeneration.

2. Material and methods

2.1. Approach to the systematic literature review

We performed a systematic review of the literature. The Medline databases Pubmed and Pubmed central, as well as CINAHL, Embase, Google Scholar and Science Direct were searched. The main search terms included Spinal Cord Injury, Intravital Imaging, Intravital Microscopy, Magnetic Resonance Imaging (MRI), Ultrasound, Photoacoustic Imaging, Laser Speckle Imaging, and Micro-Computed Tomography (μCT), in combination with Spinal Cord Injury and rodent subjects. The search included no time limit. Articles in English were included, meeting the following criteria: experimental research, original studies, rodent subjects, intravital imaging including MRI, μCT, Intravital Microscopy, Laser Speckle Imaging or Ultrasound techniques. The systematic literature search was conducted independently by four researchers from March 2020–November 2021. Data analysis was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. Obtained for review were 689 articles, of which 492 were sorted out after screening due to not meeting the inclusion criteria. An additional 104 articles were excluded due to not meeting the inclusion criteria after full-text assessment for eligibility. For qualitative synthesis 93 articles were assessed in detail and included in this publication (Fig. 1: PRISMA flow chart).

2.2. Relevant experimental in vivo imaging methods according to the literature search

According to the systematic literature search and the inclusion of 93 articles for qualitative data synthesis, five main in vivo imaging techniques in use in experimental SCI research were identified: 1. Magnetic Resonance Imaging with functional (f)MRI and microstructural MRI, 2. Micro-CT (μCT), 3. Laser Speckle Contrast Imaging (LSCI), 4. Ultrasoundography with Very High Resolution Ultrasound (VHRUS) and Photoacoustic Imaging (PA), and 5. Intravital Microscopy including Epifluorescence Videomicroscopy (IVM) and 2- or Multiphoton-Microscopy (TPEF). In the following, we discuss these topics in detail, including history, technical development, area of applications, strengths and weaknesses and potential outlook.

2.3. Illustrative examples of in vivo imaging

In Figs. 2–4, we display exemplary images of in vivo imaging methods.
performed at our institutions in murine specimens (C57BL/6J) with SCI or sham injury. The illustrative images were produced as by-products during the performance of experimental SCI studies conducted at our local institutions and were not previously published. All animal procedures were approved by the local governmental institutions (G0314/17). A continuous and close monitoring protocol was followed, and potent pain medication was applied as described in detail before (Subbeyard et al., 2014; Figley et al., 2014; Forgione et al., 2017). For SCI induction, the Clip Compression Contusion Injury model was used and for sham injury a two-level laminectomy without SCI was performed at the thoracic level (T6/7 or T10/11) (Joshi and Fehlings, 2002). For imaging procedures, volatile anesthesia (isoflurane; for magnetic resonance

Fig. 2. In vivo T2 TurboRARE 7 T Magnetic Resonance Imaging in mice with Sham-injury and SCI for axial and sagittal imaging of the spinal cord (spinal cord swelling after SCI, green arrows). Ex vivo MRI (spinal cord in saccharose) following sacrifice is also possible with exact volumetry of grey and white matter as well as injury volumetry (in blue/green, right). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3. A Technical Setting of in vivo Very High Resolution Ultrasound (VHRUS) in a murine model of experimental SCI. B Example of VHRUS of the intact (Sham) and injured (SCI) murine spinal cord in B-mode and Power Doppler mode for structural, functional and volumetrical analysis of SCI. The white arrows show structural alterations in a specimen with SCI. The images of VHRUS in B-Mode and Power Doppler Mode are printed with the permission of Michael G. Fehlings and Anna Badner.
imaging, MRI) or intravenous anesthesia (Ketamine, Xylazine; for Epi-
fluorescence videomicroscopy and very high resolution ultrasound,
VHRUS) was used. MRI was performed using a 7 T small-animal MRI
scanner (7 T PharmaScan 70/20USR, Bruker corp.). For VHRUS, the
VEVO 770 (FUJIFILM VisualSonics) ultrasound system was used (Sou-
beyrand et al., 2014a; Badner et al., 2016). For longitudinal
in vivo
microscopy, an implanted spinal window chamber adapted from Farrar
et al. was implanted at the thoracic level following SCI or sham injury
(Th10/11) and epi
fluorescence videomicroscopy was performed in the
spinal cord as described before in the brain (Farrar et al., 2012; Vajkoczy
et al., 1999; Uhl et al., 2018).

3. Theory

With this study we aim to comprehensively review experimental
setups using in vivo imaging of different kind in SCI, to give an up-to-date
overview to experimental researchers dealing with spinal cord regener-
ation and repair following SCI. In the following sections, we will review
in vivo imaging techniques used in modern experimental settings to
analyze SCI and spinal cord regeneration in detail.

4. Results

4.1. Structural and functional magnetic resonance imaging (MRI/fMRI)

4.1.1 History: Magnetic resonance imaging (MRI) and its derived
techniques are the oldest of the imaging techniques discussed, with the
underlying methods originating between the 1980s and early 2000s
(Falconer et al., 1994; Bass et al., 1994; Malisza and Stroman, 2002;
Ford et al., 1994; Ramu et al., 2006). The clinical use of MRI predates its
use in animal experimental research. MRI as a tool to image experimental
SCI in rodents was first established by Hackney et al., in 1986 (Ford et al.,
1994). Falconer et al. in 1994 were the first to perform a longitudinal in
vivo imaging study to quantify SCI pathology using MRI by constructing
3D-volume rendered images (Falconer et al., 1994). Within the first 5–10
years following the establishment of MRI in experimental SCI, the
additional use of diffusion tensor imaging (DTI) appeared, though largely
applied ex vivo (Cohen et al., 2009; Patel et al., 2016). Since 2002,
factual MRI (fMRI) is established in experimental SCI, with its
first application in the cervical spine in 2006 (Malisza and Stroman, 2002).
Since the beginning of its use in animal experiments, MRI quality
improved from 1,4 T to about 7–9 T, while in certain cases even
ultra-high field resolution MR imaging is available at 16,4 T (Ford et al.,
1994; Schwartz and Hackney, 2003; Brennan et al., 2013).

4.1.2 Technical: MRI scanners used for experimental imaging are not
notably different to those used in the clinical setting, though with smaller
bore sizes (30–90 cm) and an appropriately smaller surface coil. For
improving image quality during in vivo imaging, implanted coils
appeared at the time, but were abandoned later for their invasiveness
with the technical evolution of modern MRI scanners (Ford et al., 1994;
Sundberg et al., 2010). Modern experimental MRI scanners can monitor
breathing, and use respiratory and cardiac gating to reduce imaging ar-
tifacts produced by movement (Ford et al., 1994; Sundberg et al., 2010).
At the beginning mostly T1 weighted imaging with or without
contrast-enhancing and T2 or Rapid Acquisition with Relaxation
Enhancement (RARE) sequences were used (Malisza and Stroman, 2002;
Song et al., 2018; Sundberg et al., 2011). During the technical evolve-
ment, Diffusion Tensor Imaging (DTI) became predominant, using

Fig. 4. Experimental setup of in vivo microscopy of the spinal cord via implanted spinal window chamber, adapted by Farrar et al. (A + B) In vivo video-
epifluorescence microscopy via the implanted spinal window chamber at 7 days post implantation in a healthy specimen, with the arrow showing intact spinal
cord vessels (C). Schematic setup of TPEF (Two-photon excitation fluorescence microscopy) in a murine model of experimental SCI using the spinal window chamber
for longitudinal in vivo imaging (D).
computer assistance to reconstruct axonal bundles on the diffusion of present water molecules in the tissue (Basser et al., 1994; Soares et al., 2013). Functional MRI (fMRI) uses Blood Oxygen Level Dependent (BOLD) contrast changes to detect active regions containing ascending and descending sensorimotor pathways in resting state or upon stimulation (Malisza and Stroman, 2002; Ramu et al., 2006; Ghosh et al., 2009a; Ogawa et al., 1990). Specialized devices for small animals have been improved over the years to generate excellent resolution images with high contrast between grey and white matter. A higher magnetic field strength than in the clinical setting allows a stronger signal and reduces voxel size (Bilgen, 2012), with field strengths up to 16.4 T available (Brennan et al., 2013). Additionally, size-adjusted volume and surface coils achieve noise reduction and decrease the field of view for a higher signal to noise ratio (Bilgen, 2012). However, with varying field strength and for each imaging modality mentioned above the common acquisition parameters must be adjusted, which include but are not limited to repetition time, echo time, field of view and slice thickness (Bilgen, 2012).

4.1.3 Areas of application: MRI in SCI research is mostly used for the morphologic assessment of white matter pathology, including volumetric calculations of the lesion, and labeling of hyper- and hypointense areas as edema, hemorrhage or necrosis (Brennan et al., 2013; Sundberg et al., 2010; Song et al., 2018). The progression of the lesion volume over time can be assessed, displaying pathophysiologic changes at both the epicenter and at areas rostral and caudal of the lesion, allowing for a prediction correlation to neurologic function (Wilkins et al., 2020). Following SCI, the integrity and regeneration of the Blood-Spinal-Cord-BARRIER (BSCB) can also be assessed with MRI (Sundberg et al., 2011; Bilgen et al., 2001; Patel et al., 2009). Using Diffusion Tensor Imaging (DTI) technique, axonal integrity can be assessed (Tu et al., 2013). Another interesting area of application is the assessment of cortical plasticity following SCI using functional MRI (fMRI) (Ghosh et al., 2009a; Matsubayashi et al., 2018). This technique holds the ability to display the redistribution of somatosensory neurons in the cortex featuring the rewiring of hindlimb motoneurons (Ghosh et al., 2010). As a correlating technique Transcranial Magnetic Stimulation (TMS) has been shown to influence cortical plasticity, with both electromyography (EMG) and high-resolution MRI detecting cortical responses to hindlimb stimulation in SCI animals, delivering promising results on enhancing neuronal plasticity and functional recovery (Krishnan et al., 2019). Additionally, MRI allows for the dynamic and non-invasive monitoring of stem cell migration after transplantation (Filippi et al., 2016; Gonzalez-Lara et al., 2011; Hu et al., 2012a; Shen et al., 2009). A selection of contrast agents to render stem cells MRI-detectable have been tested in rodent SCI models, most frequently superparamagnetic iron-oxide particles (SPION) (Hu et al., 2012a; Shen et al., 2009; Gonzalez-Lara et al., 2009) and more recently Gadolinium-based (Gd) (Filippi et al., 2016; Yahyapour et al., 2018). While both show promising images of initial cell movements, signal loss is observed after about ten days with Gd agents, likely due to dilution from continuous cell division, as all contrast agents are administered in vivo (Filippi et al., 2016). SPION signals were detectable up to three weeks post injection by Hu et al. (2012), but more susceptible to artifacts (Hu et al., 2012a). Guzman et al. (2007) have managed to trace SPION-labeled stem cells for up to 18 weeks, however in the brain (Guzman et al., 2007).

4.1.4 Strengths and weaknesses: A good correlation of white matter integrity and regeneration with functional recovery could be demonstrated in studies using MRI, neurobehavioral analysis and histopathology (Wilkins et al., 2020). The rodent’s physiological movements remain a problem, causing longer examination times as compared to humans (Schilling et al., 2019). Rodent studies therefore often use a small sample size, due to high cost and effort (Ramu et al., 2006; Filippi et al., 2016; Tu et al., 2010). To increase availability and reduce material costs, the use of clinical 1.5–3 T MRI scanners in rodents has been investigated, most recently by Derksen et al. (2021) The authors performed fMRI on rats with a custom-designed coil in a clinical scanner. However, resulting voxel size and repetition time were inferior to specialized animal scanners (Derksen et al., 2021). Small animal scanners with a higher range of field strengths allow the imaging of spinal cord tissue in growing detail. Thus, contrast-labeled stem cells in the literature were traceable with 4.7 and 7 T scanners (Filippi et al., 2016; Gonzalez-Lara et al., 2011), whereas fMRI of neuronal pathways could be carried out at 9.4 T (Ghosh et al., 2010), and the exact location of responses to stimulation using targeted TMS therapy was evaluable by 11.1 T MRI (Krishnan et al., 2019). Clear differentiation of white and grey matter in the rodent spinal cord, including pathologic changes, is indeed possible in clinical 2 T scanners - as was demonstrated as early as 1994 by Falconer et al. in the first longitudinal in vivo MRI study of murine SCI (Falconer et al., 1994). However, DTI allows quantitative assessment of white matter integrity, and Brennan et al. suggest that 16.4 T DTI detects changes in radial diffusivity better than a similar study carried out at 4.7 T, proposing a higher field strength beneficial (Brennan et al., 2013). MR images are stationary and cannot monitor dynamic changes in real-time, like in imaging methods discussed below (Breiu et al., 2010; Richards et al., 2017; Najazadeh et al., 2020; Mallidi et al., 2011; Luke et al., 2012; Vawda et al., 2019; Badner et al., 2019). However, MRI still remains one of the most familiar and well-established imaging modality and is widely available (Farrar et al., 2012).

4.1.5 Outlook: Longitudinal in vivo rodent MRI provides the potential to explore microstructural damage and regeneration of the spinal cord following SCI (Duval et al., 2015). With DTI, axonal integrity and remyelination can potentially be assessed (Tu et al., 2013), while cortical plasticity following SCI can be examined using fMRI (Ghosh et al., 2009a; Matsubayashi et al., 2018) (Study overview: Table 1 (Patel et al., 2016; Brennan et al., 2013; Ghosh et al., 2010; Filippi et al., 2016; Endo et al., 2007; Ghosh et al., 2009b), Fig. 2: Examples of in and ex vivo MRI and volumetry in SCI).

4.1.6 Potential for clinical translation: As clinical scanners predate specialized small animal scanners, availability does not pose an obstacle. Monitoring the development of the BSCB in patients may provide insight into an opportune moment for therapeutic intervention, while fMRI will help in understanding changes to ascending and descending pathways that can be targeted (Bakhsheshian et al., 2021).

4.2. Micro-Computed Tomography

4.2.1 History: In 1994, Micro-Computed Tomography (μCT) appeared in experimental use. Initially mostly used for high-resolution bone imaging ex vivo, the method found its application in experimental SCI rather recently (Xu et al., 2017; Hu et al., 2012b, 2015; Tschuchnig et al., 2021; Cao et al., 2017; Zambrano-Rodríguez et al., 2019). Although in SCI research μCT is mostly used ex vivo too (Xu et al., 2017; Hu et al., 2012b, 2015; Cao et al., 2017; Strotton et al., 2021), recent studies started with in vivo protocols to assess Spinal Subarachnoid Space (SSAS), or to monitor microangiography and posttraumatic tissue integrity, as well as stenosis of the spinal canal (Zambrano-Rodríguez et al., 2019, 2021a; Lee et al., 2012; Huang et al., 2020).

4.2.2 Technical: With μCT, a high-speed full-body scan can be performed in a small animal model with ultra-low X-ray radiation doses, with less than 2 mGy whole-body in a mouse. The technical background is the illumination of a sample with a micro-focus X-ray source. Through rotation and serial imaging acquiring 2D images, later 3D reconstruction and thus the complete imaging of a sample at high resolution can be achieved. This way in SCI, 3D-imaging of the micro-neurovascular anatomy or imaging of the SSAS can be achieved at a very high resolution, in vivo reported by Zambrano-Rodríguez et al. (2019) as 100 μm in rats (Hu et al., 2012b, 2015; Zambrano-Rodríguez et al., 2019, 2021a, 2021b; Cheng et al., 2015).

4.2.3 Areas of application: In SCI research, μCT is most frequently used ex vivo to 3D image explanted specimen (Hu et al., 2012b, 2015; Cao et al., 2017). One large field of use is the high-resolution imaging of bone quality in SCI induced sarcopenia (Otzel et al., 2019; Wu et al., 2021;
**Table 1**

Exemplary articles to the experimental usage of MRI and fMRI, Ultrasound, and in vivo Microscopy after Spinal Cord Injury.

| Author (Date)         | Animal                  | Injury model | Injury location | Imaging method | Primarily analyzed issue                      |
|-----------------------|-------------------------|--------------|-----------------|----------------|-----------------------------------------------|
| Brennan et al. (2013) | C57BL/6 mice            | Contusion    | Thoracic, Th9    | MRI, DTI       | White matter pathology and regeneration       |
| Endo et al. (2007)    | Sprague-Dawley rats     | Transection  | Thoracic, Th9    | MRI, DTI       | Cortical rewiring                              |
| Filippi et al. (2016) | Balb/c mice             | Lateral      | Lumbar, L2      | MRI, DTI       | Tracking of Gadoteridol-labeled Mesenchymal stem cells |
| Ghosh et al. (2009a, 2009b) | Lewis rats          | Lateral      | Cervical, C4    | BOLD fMRI      | Cortical rewiring                              |
| Ghosh et al. (2010)   | Lewis rats              | Transection  | Thoracic, Th8    | BOLD fMRI      | Cortical rewiring                              |
| Patel et al. (2016)   | Sprague-Dawley rats     | Contusion    | Thoracic, Th7    | MRI            | BSCB permeability                              |
| Zambrano-Rodríguez et al. (2019, 2021a) | Sprague-Dawley rats | Compression | Thoracic, Th6-Th10 | Ultrafast CEU | Hemodynamic changes                            |
|                       |                         | Clip Compression | Thoracic, Th10-Th12 | VHRS | Vascular injury and regeneration               |
| Kiang et al., 2018    | Sprague-Dawley rats     | Irradiation  | Lumbar, L2-L3    | VHRUS          | Vascular injury and regeneration               |
| Soobeeyrand et al. (2014a, 2014b) | Athymic nude/C57BL/6 mice | Pinprick | Thoracic, Th11   | TPEF           | Anoxia of the dorsal funiculi                  |
|                       |                         |              |                 |                |                                               |
| In vivo microscopy    |                         |              |                 |                |                                               |
| Kerschensteiner et al. (2005) | C57BL/6 mice | Pinprick | Cervical, C3-C6 | WFFM           | Axonal degeneration and regeneration           |
| Farrar et al. (2012)  | C57BL/6 mice (YFP-H)    | Laser ablation | Thoracic, Th12  | TPEF           | Axonal degeneration and scar formation         |
| Fenrich et al. (2013) | C57BL/6 mice           | Unilateral   | Thoracic – Lumbar, Th12 | TPEF | Infiltrating and resident myelomonocytic cells |
| Hortuchi et al. (2015) | B6.Cg-Tg (Thy1-YFP)    | Contusion    | Th11            | TPEF           | Anoxia of the dorsal funiculi                  |
| Chen et al. (2017a, 2017b) | Sprague-Dawley rats | Pinprick | Cervical, C7    | TPEF           | Vascular changes                               |

**Abbreviations:** BOLD = Blood Oxygen Saturation Level, BSCB = Blood-Spinal-Cord-Barrier, C = Cervical vertebra, CEU = Contrast-Enhanced Ultrasound, DTI = Diffusion Tensor Imaging, fMRI = functional Magnetic Resonance Imaging, I = Lumbar vertebra, MRI = Magnetic Resonance Imaging, PA = Photoacoustic Imaging, Th = Thoracic vertebra, TPEF = Two Photon Excitation Fluorescence Microscopy, VHRUS = Very High Resolution Ultrasound, WFFM = Widefield Fluorescence Microscopy, YFP = Yellow Fluorescent Protein.

Yarrow et al., 2014; Debaud et al., 2017). *In vivo*, Zambrano-Rodríguez et al. 2019 were able to combine myelography with contrast-enhanced intravital μCT to image the SSAS in the rat and were later able to provide high-resolution *in vivo* images of changes in the SSAS following SCI (Zambrano-Rodríguez et al., 2019, 2021b). Moreover, μCT is used for the assessment of spinal canal width in a rodent model of spinal spondylotic myelopathy (Lee et al., 2012) and for the imaging of spinal cord integrity in therapy studies following SCI (Huang et al., 2020).

**4.2.4 Strengths and weaknesses:** In the *ex vivo* application, one strength of μCT is its non-destructiveness and therefore the preservation of samples for later destructive analyses like histology (Hu et al., 2012b, 2015; Cao et al., 2017). In vivo, the potential for high-resolution SSAS imaging has been shown. Also, tissue integrity following injury and the regeneration potential of SCI therapies was examined (Zambrano-Rodríguez et al., 2019, 2021b). In comparison to MRI, μCT image acquisition can be fast, but high-resolution 3D image reconstruction takes up to 5 h (Zambrano-Rodríguez et al., 2019, 2021a) and a complex IT infrastructure for image processing is necessary. Although μCT uses low radiation doses, repeated in vivo full body imaging of small animals can lead to relevant radiation exposure (Zambrano-Rodríguez et al., 2021b).

**4.2.5 Outlook and potential for clinical translation:** In addition to the beforementioned, interesting is the ability of modern μCT scanners to be combined with PET imaging and thus the multiplicity of possible experimental applications in vivo. In the clinic, μCT is reserved for high-resolution *ex vivo* sample analysis, for example in breast cancer and pulmonary diseases (Mai et al., 2017; Dicorpo, Tiwari, Tang, Griffin, Afreih, Pinky Bautista-Hughes, Gershfenfeld, Michaelson).

**4.3. Laser Speckle Contrast Imaging**

**4.3.1 History:** The fundamental principle of Laser Speckle Contrast Imaging (LSCI) evolved in the 1960s and 1970s (1976; Briers et al., 2013). Later on, it has been used for real-time imaging of blood flow mostly on the surface of the cortex in neuroscience and clinical research (Briers et al., 2013). Its usage for experimental application in SCI up to date is limited.

**4.3.2 Technical:** In LSCI, a laser light (coherent light, infrared or near infrared) throws light on an object with an irregular surface which is then reflected. This creates an interference pattern, called a speckle (Briers et al., 2013; Briers, 2001; Davis et al., 2014). The speckle approach was originally a single point measurement method; later scanning techniques were developed to provide a map of velocities (Briers et al., 2013; Briers, 2001). The theory of laser speckle contrast analysis is based on the principle that motion causes alterations in the speckle pattern, as for the movement of blood cells. Fast flow leads to a more indistinct pattern while the contrast is diminished whereas a decrease in flow is associated with higher contrast (Briers, 2001; Hecht et al., 2009; Lesage et al., 2009). Cameras detect the speckle pattern, and contrast can be calculated. Based on that, contrast calculating software is able to create a color-coded map for example of blood flow velocities (Hecht et al., 2009; Senarathna et al., 2013). However, imaging depth remains low, creating a momentary map of surface vascular anatomy (Senarathna et al., 2013; Gallagher et al., 2019). Depending on the wavelength the tissue can be penetrated by less than 1 mm and a spatial resolution of about 10 µm can be achieved (Senarathna et al., 2013).

**4.3.3 Areas of Application:** LSCI is mostly used to map superficial vessels in the cortex and less to image deep vessels in the spinal cord (Senarathna et al., 2013). Thus, LSCI is rarely used in experimental spinal cord imaging compared to its wide usage in measuring the blood flow of the cortex (Lesage et al., 2009; Dunn et al., 2005; Luo et al., 2007; Woitzik et al., 2013). Reason for this is mostly the limited depth of this technique and the challenge of respiration artifacts (Lesage et al., 2009). However, some SCI researchers used this tool to measure the restoration of spinal cord blood flow following electrical stimulation, or for imaging the superficial vascular reaction to neuronal stimulation in injured and non-injured rodents (Lesage et al., 2009; Beaumont et al., 2014; Brieu et al., 2010).

**4.3.4 Strengths and Weaknesses:** LSCI is a non-invasive, real-time technique which enables full-field imaging in 2-dimension. The equipment is well accessible (common laser, optics, and camera) at relatively low cost. An additional contrast agent is not required (Briers, 2001; Richards et al., 2017; Murari et al., 2007). Although the spatial and temporal resolution of this imaging technique is high, imaging depth remains low, making the technique not suitable for imaging deeper...
vascular networks in the spinal cord (Lesage et al., 2009; Senarthna et al., 2013).

4.3.5 Outlook and potential for clinical translation: Although widely used in the clinic and in the laboratory for cortical surface blood flow analysis, the use of LSCI for clinical and experimental application in SCI remains limited.

4.4. Very high resolution ultrasound and Photoacoustic Imaging

4.4.1 History: The use of Very High Resolution Ultrasound (VHRUS) as an in vivo imaging tool in SCI has emerged over the last decade. With a trend towards the use of higher frequency ultrasound, a higher spatial resolution can be achieved. Moreover, a combination with other techniques like contrast-enhanced Ultra-sonography and Photoacoustic Imaging (PA) is possible (Xu and Wang, 2006). This makes VHRUS an inexpensive and user-friendly tool for in vivo imaging of the whole spinal cord following SCI (Soubeyrand et al., 2012, 2014b; Figley et al., 2013a).

4.4.2 Technical: VHRUS is possible with specialized ultrasound machines (Soubeyrand et al., 2014a, 2014b). Using a high frequency (40–50 MHz), high resolution with 20–30 microns per pixel can be achieved (Soubeyrand et al., 2012; Finn-Bodner et al., 1995; Huang et al., 2013; Jones et al., 2012; Dubory et al., 2015). Contrast-enhanced ultrasound techniques is possible via the intravenous administration of microbubbles serving as a contrast-agent (Soubeyrand et al., 2012; Dubory et al., 2015). Compared to other functional vessel imaging like MR-angiography or CT-angiography, emerging ultrasound techniques allow for a higher resolution and co-registering with Photoacoustic Imaging (PA) is possible (Khaing et al., 2018). PA is based on the physical principle of the photoacoustic effect, initially reported in 1880. With this technique, non-invasive image-based oxygen saturation measuring in spinal cord vessels and spinal cord tissue is possible to assess hypoxia and ischemia (Xu and Wang, 2006; Figley et al., 2013b; Bell, 1880). In PA, absorption by the target molecule leads to a local temperature rise and therefore to thermal expansion of the tissue producing acoustic waves recognized by an ultrasound transducer (Najafzadeh et al., 2020; Mallidi et al., 2011). Distinct absorption properties apply to various optical chromophores (i.e. oxygenated or deoxygenated hemoglobin, melanin, water, and lipid) (Jeon et al., 2016). An additional combination with contrast agents like contrast dyes or nanoparticles is possible to increase the photoacoustic signal (Xu and Wang, 2006; Luke et al., 2012). With appropriate contrast agents, imaging on the cellular and even molecular level is realizable (Najafzadeh et al., 2020; Mallidi et al., 2011; Jeon et al., 2016). Also, the technique allows for 3D tissue imaging (Soubeyrand et al., 2014a; Moonen et al., 2016; Vawda et al., 2019).

4.4.3 Areas of Application: In experimental SCI, VHRUS can be used to longitudinally quantify the posttraumatic morphological alterations as well as changes in hemodynamics in the spinal cord (Moonen et al., 2016; Badner et al., 2019). Parameters thus assessable include lesion volume, cavity volume, parenchymal hemorrhage and alterations in spinal cord blood flow (Soubeyrand et al., 2014b; Dubory et al., 2015; Khaing et al., 2018). Additionally, the migration of infused mesenchymal stem cells can be examined (Vawda et al., 2019; Soubeyrand et al., 2014c). In combination with PA, oxygen saturation of the spinal cord tissue and vasculature can be non-invasively assessed (Figley et al., 2013b; Soubeyrand et al., 2014c). Thus, using VHRUS the effects of potential neuroprotective and regenerative treatments such as norepinephrine and mesenchymal stem cells could previously be assessed (Vawda et al., 2019; Soubeyrand et al., 2014c) and the role of IL-10 in the vascular pathology of SCI could be examined (Badner et al., 2019).

4.4.4 Strengths and Weaknesses: VHRUS allows for low-invasive, real-time longitudinal in vivo 3D-imaging of the whole spinal cord with high resolution and high depth (Vawda et al., 2019; Soubeyrand et al., 2014c). Some investigators perform repetitive surgery for VHRUS application on top of the dura. However, more modern technique allows the non-invasive application of VHRUS through the skin. Using ultrasound, tissue morphology is preserved and unaltered post mortem tissue processing remains possible (Soubeyrand et al., 2014b; Moonen et al., 2016). Apart from the VHRUS machine, the technique is inexpensive, with manageable resources necessary. Image acquisition is fast and can easily be adjusted (Soubeyrand et al., 2014b; Dubory et al., 2015). PA and contrast-enhanced imaging can be combined, allowing for accurate and rapid monitoring of effects of prospective treatments targeting spinal cord regeneration (Soubeyrand et al., 2014a, 2014c; Dubory et al., 2015; Vawda et al., 2019).

4.4.5 Experimental outlook: VHRUS is a fast and easily adjustable technique for longitudinal 3D in vivo imaging of the whole spinal cord (Soubeyrand et al., 2014a). The combination with contrast-enhanced ultrasonography allows for the tracking of blood flow and for cell tracking, whereas the combination with PA allows for non-invasive oxygen saturation measurement in the spinal cord and spinal cord vasculature (Soubeyrand et al., 2014a; Figley et al., 2013b). The trend with ultrasound techniques goes towards higher frequencies leading to even higher resolution (Dubory et al., 2015) (Study overview: Table 2 (Soubeyrand et al., 2014a; Khaing et al., 2018; Figley et al., 2013b), Schematic setup of VHRUS and exemplary images in B-Mode and Power Doppler Mode: Fig. 3).

4.4.6 Potential for clinical translation: To monitor spinal cord blood flow following injury in humans with minimally-invasive ultrasound techniques is of interest (Chen et al., 2017a; Khaing et al., 2020), as the hemodynamic stability and an intact perfusion of the spinal cord is associated with an improved outcome regarding functionality (Wermdle et al., 2014; Tator, 1991). Another potential translational approach to integrate ultrasound in combination with specific contrast agents could allow molecular imaging in clinical SCI (Khaing et al., 2020). In a pre-clinical model targeted contrast-enhanced ultrasound (TCEUS) was already described as a promising tool for the future (Volz et al., 2016, 2017).

4.5. Spinal cord microscopy

4.5.1 History: The first experimental tool used for in vivo microscopy was wide field fluorescence microscopy (WFFM), also called in vivo Epifluorescence Videomicroscopy (IVM), a basic microscope technique to image fluorescence (Kerschensteiner et al., 2005; Misgeld et al., 2007; Vajkoczky et al., 2001). However, due to limited penetration depth and limited axial resolution, the technique developed further. Two-photon excitation fluorescence microscopy (TPEF) was established in 1990, and first used in animal experiments to image glial cells in the brain (Helmchen and Denk, 2005; Denk et al., 1990). Later, TPEF was used for the tracking of immune cells in various organs. After many ex vivo studies it was first used in vivo 2003 for the imaging of lymphocytes (Cahalan et al., 2003). Originally only used for imaging cell movement, TPEF was later on also used with fluorescent proteins to image cell functions in vivo (Kavakami, 2018). Up to date, TPEF has become one of the superior tools for in vivo imaging.

4.5.2 Technical: In vivo imaging of the spinal cord is possible following surgical removal of the laminae (laminectomy), and for longitudinal in vivo imaging can be combined with the implantation of a spinal window chamber (Farrar et al., 2012; Figley et al., 2013b; Fenrich et al., 2012). In Epifluorescence Videomicroscopy (IVM), the specimen is exposed to light while illumination and detection of light cover the whole visual field simultaneously. The light source can be an LED, Mercury or Xenon arc-lamp, combined with an optical filter to choose wavelength. The image is captured by camera. Out of focus light and diffraction-limited optics result in comparably low contrast. Therefore, layers of single cells or organelles are best suited for this imaging modality (Sanderson et al., 2014). Two-photon excitation fluorescence microscopy (TPEF) is a non-linear optical method, based on photon excitation. Two photons of the same wavelength excite a fluorophore simultaneously. Together, they create enough energy for the emission of a single fluorescence photon with a higher level of energy and with shorter wavelength. This represents one pixel in the image. A
and/or GFP (green fluorescent protein). The use of sophisticated spinal window chambers. A magnitude of the possibility for longitudinal imaging without repetitive surgery with the usage of sophisticated spinal window chambers. A magnitude of different fluorescent dyes and transgenic mouse lines can be combined with this imaging method. The use of fluorescent dyes coupled to molecules of different sizes (e.g. dextran) further allows for the assessment of extravasation kinetics and a detailed analysis of BSCB (blood-spinal-cord-barrier) function and integrity. Frequently used transgenic mouse lines possess promoters coupled to fluorescent proteins YFP to image motor and sensory neurons and/or GFP (green fluorescent protein) to image mononuclear cells (Horiiuchi et al., 2015). Moreover, SCaI-IREF can be used for imaging Ca²⁺-dynamics in live neurons, while Adeno-Associated Virus (AAV) expressing a fluorophore (e.g. GFP) can function as a tracer for axon-regeneration (Liao et al., 2017; Uckermann et al., 2015; Galli et al., 2018).

To assess axonal degeneration and regeneration following SCI, in vivo imaging of YFP-labeled axons allows for the visualization and quantification of acute axonal degeneration and Wallerian degeneration. The combination of TPEF microscopy with Third Harmonic Generation microscopy reveals the spatially and temporally overlapped degeneration of axons and myelin after SCI (Farrar et al., 2011a). Recently, the formation and dynamic change of spheroids has been examined, thereby further characterizing the acute axonal degeneration post injury (Orem et al., 2020a, 2020b). To further understand the pathophysiological processes underlying axonal degeneration, intracellular Ca²⁺ levels were quantified using mice containing the GFP-positive calcium indicator GCaMP6f in axons (Orem et al., 2020b; Tang, Zhang, Chen, Xinran, Ju, Liu, Gan, He, Zhang, Li, Zhang, Kirby). Some of the parameters mentioned above were also used to show the therapeutic effects of e.g. Methyprednisolone and Progesterone on axonal survival in vivo (Zhang et al., 2015; Orem et al., 2020a; Tang, Zhang, Chen, Xinran, Ju, Liu, Gan, He, Zhang, Li, Zhang, Kirby; Yang et al., 2017b). To examine vascular changes post SCI, most studies imaging vasculature via TPEF microscopy in vivo use the visualization of vessels as orientation and show the interaction with extra-axonal cells like microglia or axons (Davalos and Akassoglou, 2012; Oshima et al., 2014; Fenrich et al., 2013). Also, in vivo imaging of cell migration is possible with labeled macrophages (Farrar et al., 2012; Evans et al., 2014; Fenrich et al., 2013; Davalos, Lee, Smith, Brinkman, Ellisman, Zheng, Akassoglou).
about the multiple options available, but one possible limitation is that a more detailed review of the different subsets of every discussed imaging technique is not achieved.

Moreover, there exist multiple established experimental models for experimental SCI induction in rodents, and even between rats and mice, there exist relevant differences in SCI pathophysiology. The reviewed studies include different animal models, like weight drop injury, impactor model, clip compression contusion injury, forceps injury, hemisection, or irradiation, in mice and rats. The one specific imaging method for the one injury model or animal model is not existent and methodological heterogeneity in the analyzed studies is high. In Table 1, exemplary studies included in the data synthesis are displayed, with the information which specimen and which SCI model was used. In experimental planning, the planned use of specimen, SCI model and combined imaging method is important regarding the intended study goal. The lack of differentiation thus is another potential limitation of this review.

6. Conclusions

Experimental studies using longitudinal in vivo imaging play a significant role in the characterization of potential targets for advancing spinal cord regeneration. Techniques at hand for in vivo imaging in SCI have dynamically evolved over the past decades. With the use of advanced imaging techniques like high-resolution MRI and fMRI combined with TMS, μCT, Very High Resolution Ultrasound combined with Contrast-Enhanced or Photoacoustic Imaging, and Two-Photon Excitation Fluorescence Microscopy in combination with various fluorescent dyes and transgenic mouse lines, longitudinal real-time in vivo imaging of neuronal, vascular and cellular regeneration of the spinal cord is possible non-invasively. With this study we comprehensively review experimental setups using in vivo imaging of different kind in SCI, to give an up-to-date overview to experimental researchers striving to advance the knowledge on spinal cord regeneration and repair following SCI. A thorough knowledge of the strengths and limitations of these techniques will help to optimally exploit our current experimental armamentarium in the field.

Author's disclosure statement

No conflicts of interest exist in the submission of the manuscript and the manuscript is approved by all authors for publication.

Funding

No special funding was received for this study. VH was supported by the 2019 Integra EANS research fund and is currently funded by the BIH Charité Clinician Scientist program.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgements

PV and VH designed the study concept. VH, LM, LR and LW performed the systematic literature review and data analysis, participated in data acquisition, and wrote the manuscript. MN helped with the acquisition of the in vivo images. PV and MGF evaluated the data and revised the manuscript.

Abbreviations

μCT Micro-Computed Tomography
3D 3 Dimensional

AAV Adeno-Associated Virus
BOLD Blood Oxygen Level Dependent
BSCB blood-spinal cord barrier
CARS Coherent Anti stokes Raman Scattering
CEU Contrast-Enhanced Ultrasonography
CT Computed Tomography
CNS Central Nervous System
DRG Dorsal root ganglia
DTI Diffusion Tensor Imaging
EMG electromyography
FITC Fluorescent isothiocyanate
fMRI functional Magnetic resonance imaging
GFP Green-fluorescent protein
1H-MRS proton magnetic resonance spectroscopy
LED Light Emitting Diode
LSCI Laser Speckle Contrast Imaging
MRI Magnetic resonance imaging
MSCs mesenchymal stem cells
PA Photoacoustic Imaging
PRISMA Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RARE Rapid Acquisition with Relaxation Enhancement
RSE Rapid Spin Echo
SCBF Spinal Cord Blood Flow
SCI Spinal Cord Injury
SHG Second Harmonic generation microscopy
SNR Signal-to-noise-ratio
SSAS Spinal Subarachnoid Space
T Tesla
THG Third harmonic generation microscopy
TMS transcutaneous magnetic stimulation
TPEF two-photon excitation microscopy
VHRUS Very High Resolution Ultrasound
YFP Yellow-fluorescent protein

References

Badihwalia, J.H., Wilson, J.R., Fehlings, M.G., 2018. Global burden of traumatic brain and spinal cord injury. Lancet Neurol. 17, 24–25.
Bader, A., Yavada, R., Laliberte, A., Hong, J., Mikhail, M., Jose, A., Dragas, R., Fehlings, M., 2016. Fetal and neonatal stem cells early intravenous delivery of human brain stromal cells modulates systemic inflammation and leads to vasoprotection in traumatic spinal cord injury. Stem Cells Transl. Med. 5, 991–1003.
Bader, A., Vidal, P.M., Hong, J., Hacker, J., Fehlings, M.G., 2019. Endogenous interleukin-10 deficiency exacerbates vascular pathology in traumatic cervical spinal cord injury. J. Neurotrauma 36, 2298–2307.
Bakhsheshian, J., Strickland, B.A., Mack, W.J., Zlokovic, B.V., 2021. Investigating the blood-spinal cord barrier in preclinical models: a systematic review of in vivo imaging techniques. Spinal Cord 59, 596–612.
Basser, P.J., Mattielo, J., LeBlan, D., 1994. MR diffusion tensor spectroscopy and imaging. Biophys. J. 66, 259–267.
Beaumont, E., Guevara, E., Dubau, S., Lesage, F., Nagai, M., Popovic, M., 2014. Functional electrical stimulation post-spinal cord injury improves locomotion and increases afferent input into the central nervous system in rats. J. Spinal Cord Med. 37, 92–100.
Bell, A.G., 1880. On the production and reproduction of sound by light. Am. J. Sci. 20, 305–324.
Bilgen, M., 2012. Neuroimaging assessment of spinal cord injury in rodents. In: Animal Models of Acute Neurological Injuries II. Springer Protocols Handbooks, pp. 679–698.
Bilgen, M., Abbe, R., Narayana, P.A., 2001. Dynamic contrast-enhanced MRI of experimental spinal cord injury: in vivo serial studies. Magn. Reson. Med. 45, 614–622.
Brennan, F.H., Cowin, G.J., Kurniawan, N.D., Ruitenberg, M.J., 2013. Longitudinal assessment of white matter pathology in the injured mouse spinal cord through ultra-high field (16.4T) in vivo diffusion tensor imaging. Neuroimage 82, 574–585.
Briens, J.D., 2001. Laser Doppler, speckle and related techniques for blood perfusion mapping and imaging. Physiol. Meas. 22.
Briens, D., Dandan, D.D., Hirst, E., Kirkpatrick, S.J., Larsson, M., Steenbergen, W., Stromberg, T., Thompson, O.B., 2013. Laser speckle contrast imaging: theoretical and practical limitations. J. Biomed. Opt. 18, 066018.
Cahalan, M., Parker, I., Wei, S., Miller, M., 2003. Real-time imaging of lymphocytes in vivo Michael. Curr. Opin. Immunol. 15, 372–377.
Cao, Y., Wu, T., ding, Wu, H., Lang, Y., Li, D. zhe, Ni, S. fei, Hu, L., Bin, Hu, J.Z., 2017. Synchrotron radiation micro-CT as a novel tool to evaluate the effect of agonom-210 on cell adhesion in a rat spinal cord model. Brain Res. 1655, 55–65.

Chen, S., Smielewski, P., Czonnky, M., Papadopoulos, M.C., Saadoun, S., 2017a. Continuous monitoring and visualization of optimum spinal cord perfusion pressure in patients with acute cord injury. J. Neurotrauma 34.

Chen, G., Zhang, Y.P., Wang, W., Shi, J.B., Fu, J., J., Xi, X.M., 2017b. In vivo dual-color method for imaging vascular dynamics following convulsive spinal cord injury. J. Vis. Exp., 56565.

Cheng, X., Long, H.Q., Chen, W.L., Xu, J.H., Huang, Y.L., Li, F.B., 2015. Three-dimensional alteration of cervical anterior spinal artery and anterior radicular artery in rat model of chronic spinal cord compression by micro-CT. Neurosurg. Lett. 60, 106–112.

Colin, D.M., Patel, C.B., Abshiba Vajula, P., Sundberg, L.M., Chacks, T., Liu, S.-J., Narayana, P.A., 2009. Blood-spinal cord barrier permeability in experimental spinal cord injury: dynamic contrast-enhanced magnetic resonance imaging. NMR Biomed. 22, 332–341.

Cord, R.S., Yano, S., Kuroda, S., Lee, J., Shichinohe, H., Seki, T., Ikeda, J.U.N., Nishimura, G., Hida, K., 2005. In Vivo Fluorescence Tracking of Bone Marrow Stromal Cell, vol. 22, pp. 907–918.

Cripps, R., Lee, B., Wing, P., Weerts, E., Mackay, J., Brown, D., 2010. A global map for traumatic spinal cord injury epidemilogy: towards a living data repository for injury prevention. Spinal Cord 49, 493–501.

Davalos, D., Akassoglou, K., 2012. In vivo imaging of the mouse spinal cord using two-photon microscopy. Jove 59.

Davis, M.A., Kazmi, S.M.S., Dunn, A.K., 2014. Imaging depth and multiple scattering in two-photon laser scanning microscopy. J. Biomed. Opt. 19, 086005.

Davalos, D., Lee, J.K., Smith, B., Brinkman, B., Ellisman, M.H., Zheng, B., and Akassoglou, K. (date unknown). Stable in Vivo Imaging of Densely Populated Glia, Axons and Blood Vessels in the Mouse Spinal Cord Using Two-Photon Microscopy.

Derksen, M., Rhemrev, V., van der Veer, M., Jolink, L., Zuidinga, B., Mulder, T., Denk, W., Strickler, J., Webb, W., 1990. Two-photon laser scanning microscope for in vivo diagnosis of malignant melanoma. J. Invest. Dermatol. 104, 727–730.

Denk, W., Stricker, J., Webb, W., 1990. Two-photon laser scanning fluorescence microscopy. Science 280 (4248), 73–76.

Derksen, M., Kazmi, S.M.S., Dunn, A.K., 2014. Imaging depth and multiple scattering in two-photon laser scanning microscopy. J. Biomed. Opt. 19, 086005.

Debarbieux, F., 2009. Sensitivity and specificity of multiphoton laser tomography for in vivo and ex vivo diagnosis of malignant melanoma. J. Invest. Dermatol. 129, 1752

Duval, T., McNab, J., Setsompop, K., Witzel, T., Schneider, T., Huang, S., Keil, B., Acosta, R., 2015. Contrast enhanced ultrasound imaging for assessment of spinal cord blood flow in patients with acute cord injuries. J. Neurosurg. 124, 1227–1239.

Duan, A.K., Devor, A., Ross, P.D., 2005. Potent opioid of oxygen metabolism and hemodynamic changes during functional activation of the rat somatosensory cortex. Neuroimage 27, 279–290.

Duval, T., McNab, J., Setsompop, K., Witzel, T., Schneider, T., Huang, S., Keil, B., Kugler, J., Ewald, Y., Acosta, R., 2015. In Vivo Mapping of Human Spinal Cord Microstructure at 300 MHz. pp. 494–507.

Duan, A.K., Devor, A., Ross, P.D., 2005. Potent opioid of oxygen metabolism and hemodynamic changes during functional activation of the rat somatosensory cortex. Neuroimage 27, 279–290.

Dray, C., Rougon, G., Debarbieux, F., 2009. Quantitative analysis by in vivo imaging of the dynamics of vascular and axonal networks in injured mouse spinal cord. Proc. Natl. Acad. Sci. U.S.A. 106, 9455–9460.

Dubory, A., Laemmel, E., Bassler, A., Duranteau, J., licia, E., Curr, C., Soubyrand, M., 2015. Contrast enhanced ultrasound imaging for assessment of spinal cord blood flow in experimental spinal cord injury. J. Vis. Exp., 52536

Duval, T., McNab, J., Setsompop, K., Witzel, T., Schneider, T., Huang, S., Keil, B., Kugler, J., Ewald, Y., Acosta, R., 2015. In Vivo Mapping of Human Spinal Cord Microstructure at 300 MHz. pp. 494–507.

Duan, A.K., Devor, A., Ross, P.D., 2005. Potent opioid of oxygen metabolism and hemodynamic changes during functional activation of the rat somatosensory cortex. Neuroimage 27, 279–290.

Duval, T., McNab, J., Setsompop, K., Witzel, T., Schneider, T., Huang, S., Keil, B., Kugler, J., Ewald, Y., Acosta, R., 2015. In Vivo Mapping of Human Spinal Cord Microstructure at 300 MHz. pp. 494–507.

Duan, A.K., Devor, A., Ross, P.D., 2005. Potent opioid of oxygen metabolism and hemodynamic changes during functional activation of the rat somatosensory cortex. Neuroimage 27, 279–290.

Duan, A.K., Devor, A., Ross, P.D., 2005. Potent opioid of oxygen metabolism and hemodynamic changes during functional activation of the rat somatosensory cortex. Neuroimage 27, 279–290.

Duan, A.K., Devor, A., Ross, P.D., 2005. Potent opioid of oxygen metabolism and hemodynamic changes during functional activation of the rat somatosensory cortex. Neuroimage 27, 279–290.
Axonal Dieback, Blood Flow, and Calcium Influx with Methylprednisolone Therapy after Spinal Cord Injury.

Tator, C.H., 1991. Review of experimental spinal cord injury with emphasis on the local and systemic circulatory effects. Neurosurgery 27, 291–302.

Theer, P., Hanan, M.T., Denk, W., 2002. Two-photon imaging to a depth of 1000 μm in living brains by use of a Ti:Al2O3:20.3 regenerative amplifier. Opt. Lett. 28, 1022.

Tschauchning, M.E., Zillner, D., Romanelli, P., Hercher, D., Heimel, P., Oostingh, G.J., Couillard-Despres, S., Gadmermayr, M., 2021. Quantification of anomalies in rats’ spinal cords using autoencoders. Comput. Biol. Med. 138.

Tu, T., Kim, J., Wang, J., Song, S., 2010. Full tensor diffusion imaging is not required to assess the white-matter integrity in mouse contusion in mouse contusion spinal cord injury. J. Neurotrauma 26, 253–262.

Tu, T.W., Kim, J.H., Yin, F.Q., Jaleman, L.B., Song, S.K., 2013. The impact of myelination on axon sparing and locomotor function recovery in spinal cord injury assessed using diffusion tensor imaging. NMR Biomed. 26, 1484–1495.

Uckermann, O., Galli, R., Beiermeister, R., Sitoci-Fici, K.H., Later, R., Leipnitz, E., Neuwirth, A., Chavakis, T., Koch, E., Schackert, G., Steinier, G., Kirsch, M., 2015. Endogenous two-photon excited fluorescence provides label-free visualization of the inflammatory response in the rodent spinal cord. Biomed Res. Int. 2015.

Uhl, C., Markel, M., Broggini, T., Nieminen, M., Kremenetskaia, I., Vajkoczy, P., Czabanka, M., 2018. EphB4 mediates resistance to antiangiogenic therapy in experimental glioma. Angiogenesis 21, 873–881.

Vajkoczy, P., Goldbrunner, R., Farhadi, M., Vincze, G., Schilling, L., Tonn, J.C., Smielewski, P., Jamous, A., Anthony Bell, B., Zoumprouli, A., Papadopoulos, M.C., 2009. Two-photon microscopy as a tool to investigate the therapeutic efficacy of methylprednisolone in a rat spinal cord hemisection model. Curr. Neurovasc. Res. 14.

Yahyapour, R., Farhood, B., Grady, G., Rezaeyan, A., Rezapoor, S., Abdollahi, H., Cheki, M., Amini, P., Fallah, H., Najafi, M., Motveesan, E., 2018. Stem cell tracing through MR molecular imaging. Tissue Eng. Regen. Med. 15, 249–261.

Yang, Z., Xie, W., Ju, F., Khan, A., Zhang, S., 2017a. In vivo two-photon imaging reveals a role of progesterone in reducing axonal dieback after spinal cord injury in mice. Neuropharmacology 116, 30–37.

Yang, Z., Xie, W., Ju, F., Khan, A., Zhang, S., 2017b. In vivo two-photon imaging reveals a role of progesterone in reducing axonal dieback after spinal cord injury in mice. Neuropharmacology 116, 30–37.

Yarow, J.F., Conover, C.F., Beggs, L.A., Beck, D.T., Otzel, D.M., Balaez, A., Combs, S.M., Miller, J.R., Ye, F., Aguirre, J.L., Neuvillé, K.G., Williams, A.A., Conrad, B.P., Gregory, C.M., Wronski, T.J., Rose, P.K., Roest, S.E., 2014. Testosterone dose dependently prevents bone and muscle loss in rodents after spinal cord injury. J. Neurotrauma 31, 834–845.

Zambrano-Rodríguez, P.C., Bolanos-Puchet, S., Reyes-Alva, H.J., García-Orozco, L.E., Romero-Piña, M.E., Martínez-Cruz, A., Guizar-Sahagún, G., Medina, L.A., 2019. Micro-CT myelography using contrast-enhanced digital subtraction: feasibility and initial results in healthy rats. Neuroradiology 61, 323–330.

Zambrano-Rodríguez, P.C., Bolanos-Puchet, S., Reyes-Alva, H.J., de Los Santos, R.A., Martínez-Cruz, A., Guizar-Sahagún, G., Medina, L.A., 2021a. High-resolution micro-CT myelography to assess spinal subarachnoid Space changes after spinal cord injury in rats. J. Neuroimagiing 31, 79–89.

Zambrano-Rodríguez, P.C., Bolanos-Puchet, S., Reyes-Alva, H.J., de Los Santos, R.A., Martínez-Cruz, A., Guizar-Sahagún, G., Medina, L.A., 2021b. High-resolution micro-CT myelography to assess spinal subarachnoid Space changes after spinal cord injury in rats. J. Neuroimagiing 31, 79–89.

Zhang, Y., Zhang, L., Shen, J., Chen, C., Mao, Z., Li, W., Gan, W.-B., Tang, P., 2014. Two-photon-Excited Fluorescence microscopy as a tool to investigate the efficacy of methylprednisolone in a mouse spinal cord injury model. Spine (Phila. Pa. 1976 39, E493–E499.

Zhang, Y., Zhang, L., Ji, X., Pang, M., Ju, F., Zhang, J., Li, W., Zhang, S., He, Z., Gan, W.-B., Tang, P., 2015. Two-photon microscopy as a tool to investigate the therapeutic time window of methylprednisolone in a mouse spinal cord injury model. Restor. Neurol. Neurosci. 33, 291–300.