Journal of Neuroscience Methods 188 (2010) 39–44

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Novel use of X-ray micro computed tomography to image rat sciatic nerve and integration into scaffold

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ARTICLE INFO

Article history:
Received 13 January 2010
Received in revised form 26 January 2010
Accepted 27 January 2010

Keywords:
X-ray micro computed tomography
Rat sciatic nerve
Nerve scaffold
Osmium tetroxide
Staining
Image analysis

ABSTRACT

This paper describes how specimens of nervous tissue can be prepared for successful imaging in X-ray Micro Computed Tomography (μCT), and how this method can be used to study the integration of nervous tissue into a polymeric scaffold. The sample preparation involves staining the biological tissue with osmium tetroxide to increase its X-ray attenuation, and a technique for maintaining the specimen in a moist environment during the experiment to prevent drying and shrinkage. Using this method it was possible to observe individual nerve fascicles and their relationship to the 3-D tissue structure. A scaffold supporting a regenerated sciatic nerve was similarly stained to distinguish the nervous tissue from the scaffold, and to observe how the nerve grew through a 2.5 mm long, 100 μm × 100 μm cross-section channel polyimide array. Furthermore, blood vessels could be identified in these images, and it was possible to monitor how a large proximal blood vessel split through the channel scaffold and proceeded down individual channels. This paper explains how μCT is a useful tool both for studying the location and extent of growth into a polymeric scaffold, and for determining whether the regenerated tissue has blood supply.

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1. Introduction

Thin histological slices are traditionally used to determine the structure of biological tissue. However, these slices only provide 2-D information and their preparation can generate stresses, which may influence the tissue structure. X-ray Micro Computed Tomography (μCT) is a contactless technique that uses a tungsten-anode X-ray source and a high-resolution X-ray CCD camera (coupled to an X-ray scintillator) to obtain non-destructive, 3-D internal structural information (Holdsworth and Thornton, 2002; Bonse and Busch, 1996). Image contrast using μCT relies on X-ray attenuation differences, which depend approximately on atomic number, Z^3; consequently it is difficult to image the internal structure of tissue, and μCT is most commonly used to image bone. Nonetheless, μCT has been used to image rodent hearts, kidneys and livers (Garcia-Sanz et al., 1998; Ortiz et al., 2000; Beighley et al., 1997; Jorgensen et al., 1998) and tumours (De Clerck et al., 2004; Wan et al., 2000; Paulus et al., 2000). It has also been applied to image the microvasculature of pigs (Rodriguez-Porcel et al., 2000; Wilson et al., 2002). Ritman reviewed various techniques to improve image contrast within tissue, such as staining with heavy metal ions to increase the attenuation values; this is a common technique for tissue preparation in SEM or TEM (Ritman, 2002). Osmium tetroxide staining was found to improve the contrast of μCT images of a rat kidney (Ritman, 2002). Additionally Mizutani et al. (2007) used silver and gold stains to improve the contrast of the central nervous system of Drosophila larvae so that the neural network in slices of nerve tissue could be seen. However, one overlooked application of μCT is the ability to see how a tissue is integrating with implants. This paper describes how X-ray μCT can be used to image nervous tissue, and how this can be used to study the integration of nervous tissue into a polymeric scaffold.

2. Method

2.1. Sample preparation

3-D (three-dimensional) Polyimide (PI) nerve scaffolds were manufactured using microfabrication techniques. A detailed review of the scaffold fabrication can be found in (Lacour et

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doi:10.1016/j.jneumeth.2010.01.032

al., 2009). Briefly, the scaffolds consisted of a 2.5 mm long cylinder containing a bundle of approximately 180 longitudinal 100 μm × 100 μm channels for nervous tissue to regenerate down. Due to the design and nature of the materials used for these scaffolds, it is difficult to obtain images of the tissue content by conventional methods.

All experiments were carried out using adult Lewis strain male rats in accordance with the United Kingdom Animals (Scientific Procedures) Act, 1986.

The PI scaffolds were sterilized in 70% ethanol for 3 h. Operations were performed under anaesthesia using a mixture of intraperitoneal medetomidine (0.25 mg/kg) and ketamine (60 mg/kg). The right sciatic nerve was exposed at the thigh and freed from surrounding tissues from the sciatic notch to the knee. The sciatic nerve trunk was transected, and each nerve stump fixed into one end of the scaffold with one stitch of 10-0 suture. Implants were filled with saline. After implanting the device, the muscle and skin were reapproximated and sutured.

At 4 weeks post-implantation and under pentobarbital anaesthesia, animals were transcardially perfused with 0.9% saline solution followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), and the PI scaffold together with a section of sciatic nerve attached to each end was harvested. Nerves were postfixed in 3% glutaraldehyde overnight at 4 °C. The intact contralateral nerves were also harvested for study.

Preliminary experiments revealed that nervous tissue had a similar X-ray attenuation to water and to several polymers. To increase the X-ray attenuation of nerve tissue, staining with osmium tetroxide was performed prior to mounting. Osmium tetroxide (OsO_4) has a great affinity for lipids; it oxidises tissue fats leaving behind electron dense osmium (Hanker et al., 1966). Staining was performed by placing the glutaraldehyde fixed nerves into 2% OsO_4 for 2 h (OsO_4 penetrates into tissue by diffusion), then removing and washing in 0.1 M phosphate buffered saline (PBS) containing a small quantity of sodium azide preservative (Di Scipio et al., 2008).

At first it was attempted to prepare the control nerve specimens for μCT by drying them in air on the bench-top for 1 h and then mounting the stiffened tissue vertically in the tomograph. However, this resulted in noticeable shrinkage of the tissue (a 90% decrease in tissue cross-sectional area was typically observed) during the course of the experiments. In response to this, the following technique for wet-mounting the control nerve specimens was developed. A 2 cm section of the narrow end of a 1000 μl graduated slimline pipette tip (VWR International Ltd.) was cut, and this section was sealed at the narrow end with silicone sealant. The tip was then filled with phosphate buffered saline (PBS), the nerve was placed inside and the top of the pipette tip was sealed with silicone sealant to prevent evaporation. However, this reduced the working magnification due to the necessity of keeping the entire pipette tip in the tomograph field of view.

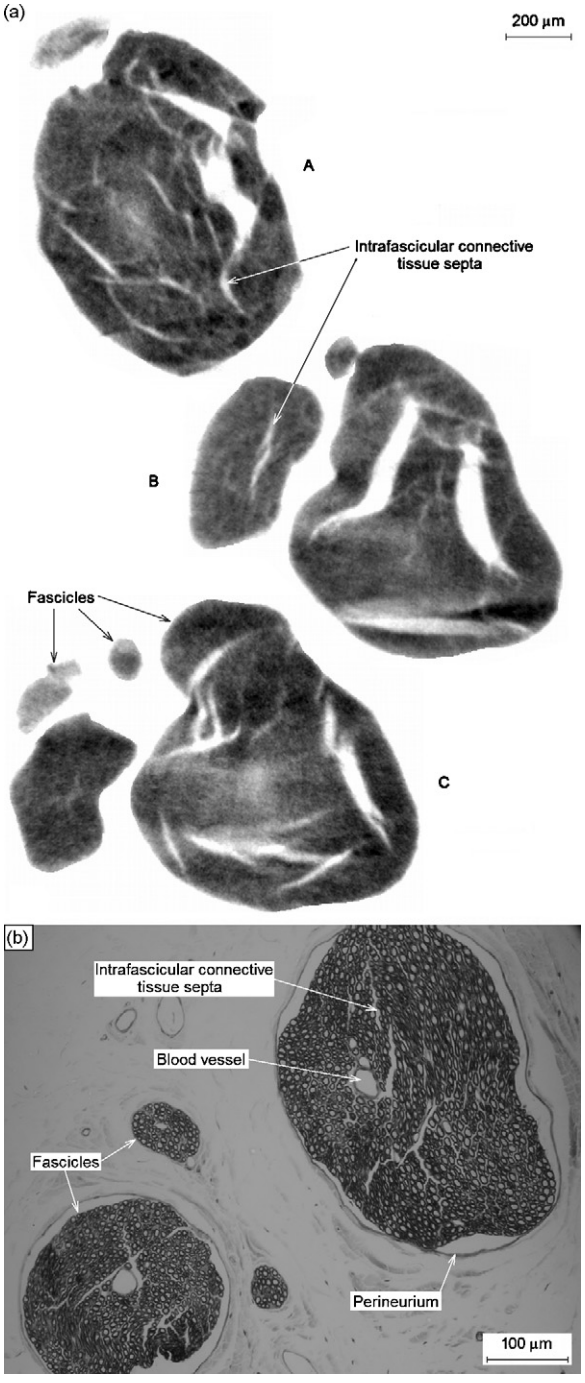

Fig. 1. (a) μCT cross-sectional slices of an osmium-stained sciatic nerve at 1 mm intervals from the proximal (A) to the distal (C) end. (b) Optical micrograph of a 5 μm transverse section of an uninjured nerve, fixed and stained with osmium in the same manner as the nerve in (a), for comparison of observable features.

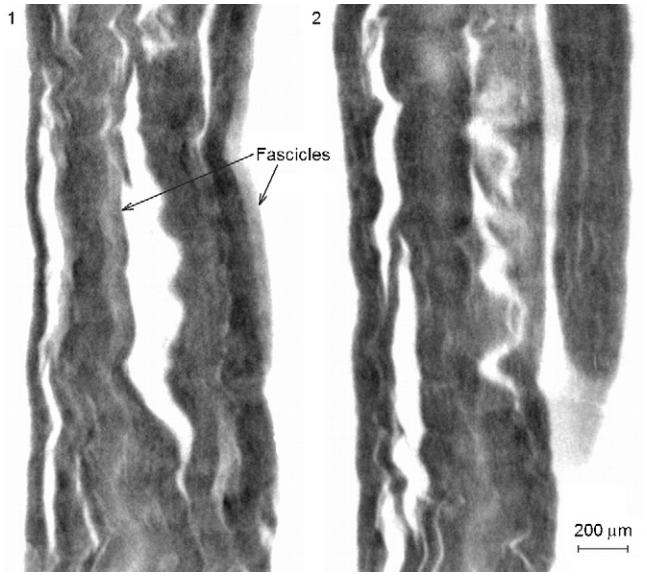

Fig. 2. Two perpendicular μCT longitudinal sections ("1" and "2") of a 2 mm section of osmium-stained sciatic nerve.

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