Journal of Neuroscience Methods 188 (2010) 258–269

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

# Establishing a model spinal cord injury in the African green monkey for the preclinical evaluation of biodegradable polymer scaffolds seeded with human neural stem cells

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ARTICLE INFO

*Article history:*
Received 7 December 2009
Received in revised form 19 February 2010
Accepted 22 February 2010

*Keywords:*
Spinal cord injury
African green monkey
Non-human primate
Stem cells
Biomaterials
Injury model
Behavioral scoring

ABSTRACT

Given the involvement of post-mitotic neurons, long axonal tracts and incompletely elucidated injury and repair pathways, spinal cord injury (SCI) presents a particular challenge for the creation of preclinical models to robustly evaluate longitudinal changes in neuromotor function in the setting in the presence and absence of intervention. While rodent models exhibit high degrees of spontaneous recovery from SCI injury, animal care concerns preclude complete cord transections in non-human primates and other larger vertebrate models. To overcome such limitations a segmental thoracic (T9–T10) spinal cord hemisection was created and characterized in the African green monkey. Physiological tolerance of the model permitted behavioral analyses for a prolonged period post-injury, extending to predefined study termination points at which histological and immunohistochemical analyses were performed. Four monkeys were evaluated (one receiving no implant at the lesion site, one receiving a poly(lactide-co-glycolide) (PLGA) scaffold, and two receiving PLGA scaffolds seeded with human neural stem cells (hNSC)). All subjects exhibited Brown-Séquard syndrome 2 days post-injury consisting of ipsilateral hindlimb paralysis and contralateral hindlimb hypesthesia with preservation of bowel and bladder function. A 20-point observational behavioral scoring system allowed quantitative characterization of the levels of functional recovery. Histological endpoints including silver degenerative staining and Iba1 immunohistochemistry, for microglial and macrophage activation, were determined to reliably define lesion extent and correlate with neurobehavioral data, and justify invasive telemetered electromyographic and kinematic studies to more definitively address efficacy and mechanism.



## 1. Introduction

Spinal cord injury (SCI) results from penetrating or compressive traumatic injury to the spine or from compressive lesions associated with neoplastic growth or vertebral dislocation. Neuronal injury and recovery is critically guided and impacted by the surrounding cells and extracellular environment within the spinal cord and adjacent tissues, reducing the utility of *in vitro* assays, and necessitating the study of injury mechanisms and spinal cord physiology in *in vivo* vertebrate models (Feringa et al., 1975; Hall and Springer, 2004; Jones et al., 2005; Liverman et al., 2005; Thuret et al., 2006; Baptiste and Fehlings, 2007; Rossignol et al., 2007). A recent review of data derived from the extensive literature related to the modeling of SCI to better understand mechanisms of injury and repair has highlighted the greater relevance and utility of non-human primate models relative to rodents and other vertebrate species in the preclinical investigation of therapeutic interventions (Courtine et al., 2007). Rodents may over-predict the efficacy of interventions given high rates of spontaneous recovery from induced spinal cord injury, even following profound lesions. The spinal cord anatomy and physiology of old world monkeys are

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doi:10.1016/j.jneumeth.2010.02.019

more similar to humans, particularly with respect to the position and function of corticospinal tracts (Courtine et al., 2007). This permits a more critical evaluation of results from preclinical studies, facilitating translation to humans. Here we report the development of a surgical model of acute SCI in the African green monkey (*Chlorocebus sabaeus*) for the evaluation of biomaterial implants as a translational interval between rodent and clinical investigations.

Poly(lactide-co-glycolide) (PLGA) biocompatible and biodegradable porous scaffolds seeded with neural stem cells (NSC) have demonstrated potential as a strategy for the treatment of central nervous system lesions (Flax et al., 1998; Park et al., 2002). A PLGA scaffold seeded with murine NSC (mNSC) promoted long-term functional improvements in an adult rat hemisection model of SCI as compared to controls (Teng et al., 2002). 70 days post-injury, treated animals exhibited coordinated, weight-bearing hindlimb stepping. Histological and immunocytochemical analysis suggested the recovery may have been associated with a reduction in tissue loss, possibly resulting from modulation of secondary injury mechanisms and reduced astrogliosis.

To establish an SCI model in which this possibility might be critically evaluated, a lateral hemisection at level T9–T10 in the thoracic spine was created in the monkey, with removal of the ipsilateral T9–T10 segment. This approach bears some similarity to previously published models, where ipsilateral tracts were transected without removal of a full segment or only particular tracts (lateral corticospinal efferents, dorsal funiculus afferents) were targeted (Crowe et al., 1997; Babu et al., 2000; Edgerton et al., 2004). The lesion was designed to result in Brown-Séquard syndrome, characterized in humans at comparable cord levels by paralysis of the ispilateral leg, loss of ipsilateral muscle tone in the lower abdomen (innervated by T9–L1), loss of vibration and position sensation in the ipsilateral hindlimb, loss of thermal and mechanical pain sensation in the contralateral leg, lumbar and sacral dermatomes and ipsilateral lower thoracic dermatomes, and spastic paresis in the ipsilateral leg resulting in increased knee and ankle jerk reflexes with retention of monosynaptic reflexes involving the lower motor neurons (Brown-Séquard, 1851). Importantly, a unilateral restriction of the lesion was implemented with the additional aim of sparing bladder function and anal muscle tone by allowing compensatory innervation by retained contralateral corticospinal efferents. Evaluation of loss and recovery of these multiple lower extremity functions may permit objective measures of the efficacy of the therapeutic intervention with minimal post-operative complications.

In addition to establishing methodologies for the generation of a discrete unilateral cord lesion designed to minimize special animal care requirements, behavioral and histological endpoints were evaluated for their utility in differentiating treatment outcomes. Neuromotor and histopathological endpoints were assessed across multiple time points following introduction of a PLGA scaffold, PLGA scaffold seeded with hNSC, or no implant.

## 2. Materials and methods

### 2.1. Hemisection model

Four juvenile male African green monkeys ranging in weight from 2.3 to 2.7 kg were employed in the study. Subjects were between 1.8 and 2.0 years old. Baseline clinical and neurological exams confirmed good health and suitability for study enrollment. Treatment allocation was performed arbitrarily with respect to weight. All experimental and surgical procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Academy Press, revised 1996) and the Institutional Animal Care and Use Committee (IACUC) of the St. Kitts Biomedical Research Foundation, where the study was conducted.

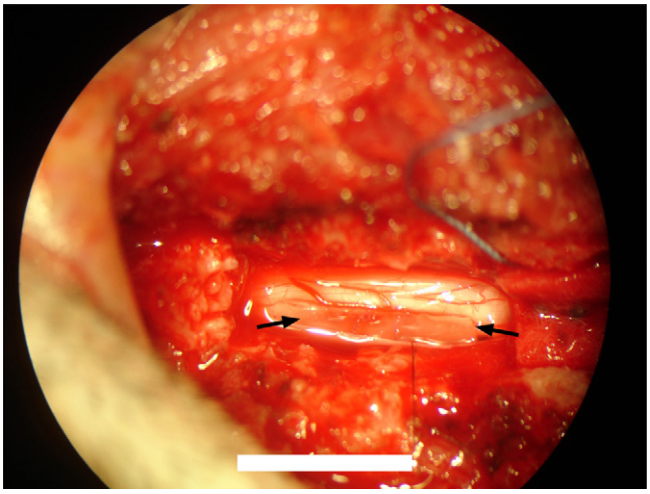

**Fig. 1.** Photograph through surgical microscope of scaffold implanted into T9–T10 hemisection lesion. Arrows indicates scaffold position. Scale bar = 10 mm.

To perform the hemisection lesion, monkeys were sedated with ketamine/xylazine i.m., and kept on a 0.9% saline drip. Isoflourane anesthesia was applied with depth of anesthesia monitored throughout the study. Deep dermal or subcutaneous bleeding was controlled using electrocautery following skin incision. The laminectomy was performed by an en-bloc, lateral lamina cut method. Following bone removal, the dural opening was performed with a #15 surgical blade. Dural edges were tacked to the surrounding ligaments or musculature with 4.0 non-absorbable sutures. The midline of the spinal cord was carefully assessed with the visualization of anatomic landmarks. At all times, spinal cord veins and surface vessels were spared unless it was absolutely necessary that they be controlled. The segmental hemisection lesion was microscopically performed with the use of microneurosurgical dissection instruments and micro-suction aspiration. A transverse incision extending to the midline was made at the caudal point of the intersection of the T9 dorsal root with the cord and at the rostral point of the intersection of the T10 dorsal root with the cord, followed by a midline incision extending between those levels. Hemi-cord parenchyma resection to these defined boundaries ensured complete transection to the anatomic midline in a lesion extending 10 mm in length, without damage to the contralateral hemi-cord. At this point in the procedure, if designated, a scaffold was implanted (Fig. 1). The scaffold was sized to fill the hemisection cavity, without exerting pressure on the surrounding host tissue during or following insertion. When this process was complete, the dural tack-up sutures were removed, and the dura was closed with a 4.0 non-absorbable single running suture. Fibrin tissue sealant was applied to the dural suture line. The fascia was then closed with interrupted 3.0 absorbable sutures. After this layer was closed, the subcutaneous layers were re-approximated with 3.0 absorbable sutures. Then, the deep dermal layer was brought together with interrupted 3.0 absorbable sutures. After successful extubation and recovery from anesthesia, a neurologic examination was performed. Vital signs were monitored in a manner consistent with standard human post-anesthesia care. Following recovery from anesthesia the monkeys were returned to their cage with a mattress placed on the floor to minimize pressure sore risk. Monkeys were observed twice daily to assess skin integrity and exclude the possibility of autophagy, which can be observed in the setting of limb denervation. All monkeys additionally received a pre and postoperative course of immunosuppressants, consisting of cyclosporine (0.6 mg/kg), prednisolone (0.3 mg/kg) and azathioprine (0.5 mg/kg) i.m. BID starting 3 days before implantation and continuing until sacrifice to prevent