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Research Report

Transplantation of artificial neural construct partly improved spinal tissue repair and functional recovery in rats with spinal cord transection

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ABSTRACT

Delivery of cellular and/or trophic factors to the site of injury may promote neural repair or axonal regeneration and return of function after spinal cord injury. Engineered scaffolds provide a platform to deliver therapeutic cells and neurotrophic molecules. To explore therapeutic potential of engineered neural tissue, we generated an artificial neural construct in vitro, and transplanted this construct into a completely transected spinal cord of adult rats. Two months later, behavioral analysis showed that the locomotion recovery was significantly improved compared with control animals. Immunoreactivity against microtubule associated protein 2 (Map2) and postsynaptic density 95 (PSD95) demonstrated that grafted cells had a higher survival rate and were able to differentiate toward neuronal phenotype with ability to form synapse-like structure at the injury site; this was also observed under the electron microscope. Immunostaining of neurofilament-200 (NF-200) showed that the number of nerve fibers regrowing into the injury site in full treatment group was much higher than that seen in other groups. Furthermore, Nissl staining revealed that host neuron survival rate was significantly increased in rats with full treatments. However, there were no biotin dextran amine (BDA) anterograde tracing fibers crossing through the injury site, suggesting the limited ability of corticospinal tract axonal regeneration. Taken together, although our artificial neural construct permits grafted cells to differentiate into neuronal phenotype, synaptogenesis, axonal regeneration and partial locomotor function recovery, the limited capacity for corticospinal tract axonal regeneration may affect its potential therapy in spinal cord injury.

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Abbreviations: SCI, spinal cord injury; PLGA, poly (lactic acid-co-glycolic acid); PLLA, poly (L-lactic acid); NTs, neurotrophins; NSCs, neural stem cells; NT-3, neurotrophin-3; TrkC, tyrosine receptor kinase C; Ad, recombinant adenoviral; MOI, multiplicity of infection; BDA, biotin dextran amine; CST, corticospinal tract; SMC, sensorimotor cortex; RN, red nucleus; CN, Clarke's nuclei

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1. Introduction

It is still a major therapeutic challenge to promote the axonal regeneration and functional recovery from spinal cord injury (SCI). The pathophysiological processes in spinal cord injury are multifactorial, involving blood vessel rupture, ischemia, edema, metabolic derangement, and free radicals formation in acute phase, and followed by axonal degeneration/regeneration, loss of glial cells, demyelination/remyelination, and formation of cavities at the injury site (Faden, 1993). It has been proposed that these devitalized tissues be replaced by artificial neural tissues in order to restore the motor functions. There have been significant developments in experimental studies of this area in the past years.

One of the strategies to repair SCI was the transplantation of neural stem cells (NSCs) (Nash et al., 2002), mainly as NSCs have the capacity to differentiate into neurons. However, grafted NSCs are rarely differentiated into mature neurons in the lesion site (Novikova et al., 2002; Sayer et al., 2002). Therefore, more and more attention has been focused on how to pre-induce NSCs differentiation in vitro.

Molecular therapies, such as administration of neurotrophins (NTs) (Fernandez et al., 1993; Namiki et al., 2000; Schnell et al., 1994), are known to play important roles in neural survival, differentiation, and neurite outgrowth (Chao, 2003; Lu et al., 2005). NSCs transfected with NT-3 gene yield a higher percentage of differentiation toward neurons (Wang et al., 2007; Park et al., 2006). This percentage may be further increased when NSCs over-expressing NT-3 are mixed with NSCs expressing tyrosine receptor kinase C (TrkC), the NT-3 receptors (Wang et al., 2007). Our previous studies revealed that gene-modified NSCs promoted axon regeneration and functional improvement when transplanted into injured spinal cord of rats, but incapable of forming a neural network to bridge the gap at the injury site (Guo et al., 2007; Lu et al., 2003; Zhang et al., 2007).

Engineered biomaterials provide a platform to deliver therapeutic cells and/or neurotrophic molecules, which have been investigated for their ability to reconstruct spinal cord tissue architecture, to provide guidance for regenerating axons, and to prevent the infiltration of scar tissue and cyst formation in the SCI animal models (Friedman et al., 2002; Lee et al., 2003; Oudega et al., 2001; Teng et al., 2002; Xu et al., 1999). Natural biomaterials and synthetic biomaterials have been used as engineered scaffolds at the injury site of experimental animals, due to the advantages that they are biodegradable and have a good biocompatibility. In addition, the degradation products are easy to be absorbed and seldom to cause inflammation. Poly (lactic acid-co-glycolic acid) (PLGA) and poly (L-lactic acid) (PLLA) polymers are representative in synthetic polymer biomaterials. For instances, PLLA scaffold-Schwann cells construct improved the hindlimb motor recovery in completely transected spinal cord model, but with lower survival rate of transplanted Schwann cells (Zhang et al., 2007). And in hemisected SCI model of rat, implantation of PLGA scaffold with NSCs into the injured site resulted in a functional improvement for one year. The transplantation reduced tissue loss and glial scarring (Novikova et al., 2002).

We recently reported that NT-3 and TrkC gene-modified neural stem cells seeded in PLGA, which generated an artificial neural construct in vitro. The artificial neural construct permits NSCs to differentiate into neurons establishing connections with each other and exhibiting synaptic activities (Xiong et al., 2009). In the present study, we investigated whether the artificial neural construct transplanted into spinal cord transected completely could still retain NSCs-derived neurons in vivo, and promote axonal regeneration and functional improvement.

2. Results

2.1. *Functional recovery*

Before SCI, all the rats showed normal locomotor performance with BBB score of 21. When spinal cord was completely transected, the hindlimbs of the animals were immediately paralyzed with BBB score of 0. Two weeks after transplantation, locomotor performance improved gradually in all experimental groups. At the end of the 8th week, scores of the Co-culture group and NT-3 group were significantly higher than other groups, and the Co-culture group exhibited an average score of 8.78±1.78, which was the highest among the groups ($P<0.05$, Fig. 1). A BBB score of 8.78 indicates that the animals were able to sweep their hindlimbs with no weight support or/and plantar paw placement with support in stance (when stationary). The 45° inclined grid test concerning limb coordination was also performed. Compared with the PLGA, NSCs and TrkC groups, the rest of groups could voluntarily place their paws on the rung and step their hindlimbs onto the grid occasionally. Especially, the Co-culture group occasionally exhibited the coordination movement of the fore-hind limbs and had more chance to step their hindlimbs onto grid (Supplementary Video 1 and 2, online),

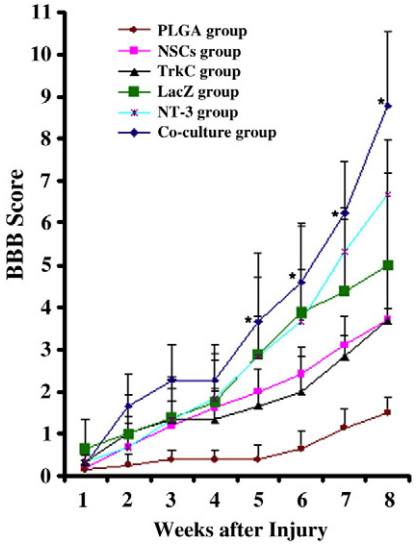

Fig. 1 – Functional recovery after injury. Basso, Beattie, and Bresnahan (BBB) scores were obtained during the course of recovery after spinal cord injury. Scores were higher in the cell-transplanted groups than that in the PLGA group; scores in the Co-culture group were the highest after the 5th week. *$P<0.05$.