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Neuroscience Letters 401 (2006) 65–70
Neuroscience Letters
www.elsevier.com/locate/neulet

Phenotypical analysis of adult rat olfactory ensheathing cells on 3-D collagen scaffolds

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Received 17 December 2005; received in revised form 13 February 2006; accepted 27 February 2006

Abstract

Olfactory ensheathing cell (OEC) transplantation is a promising or potential therapy for spinal cord injury (SCI). However, the effects of injecting OECs directly into SCI site have been limited and unsatisfied due to the complexity of SCI. To improve the outcome, proper biomaterials are thought to be helpful since these materials would allow the cells to grow three-dimensionally and guide cell migration. In this paper, we have studied the behavior of OECs in two-dimensional (2-D) condition as well as on three-dimensional (3-D) collagen scaffolds by analyzing their phenotypes such as cell proliferation, apoptosis, morphology, and gene activities of some neurotrophic factors and myelin proteins. OECs proliferation rate was increased on 3-D collagen scaffolds compared to the 2-D culture condition. OECs on 3-D collagen scaffolds also showed less apoptosis. In addition, OECs on 3-D collagen scaffolds maintained the original spindle-shape morphology and P75NTR gene activity. NGF, BDNF, and PLP were found to be upregulated in OECs cultured on 3-D collagen scaffolds by the semi-quantitative RT-PCR approach. The results suggested that 3-D collagen scaffolds provide suitable environments for the OECs to maintain their morphology as well as several important functional phenotypes and all these could be helpful for the effective treatment of SCI.

Keywords: OECs; CNS regeneration; Three-dimensional culture; Phenotype; Collagen scaffold

In spinal cord injury (SCI) repair research, there has been increasing interest in the olfactory ensheathing cells (OECs) that support neurogenesis throughout life in the olfactory system. OECs share some common properties with Schwann cells and astrocytes [6], but also have some unique characteristics that provide potential to enhance CNS axonal regeneration. In animal models, cultured OECs transplanted into the spinal cord promoted the injured neuron survival and partial functional recovery [20]. SCI, mostly caused by mechanical trauma, initiated a cascade of uncontrolled endogenous biochemical reactions and caused sequential damage at the lesion site. To reconstruct sensory and motor functions, SCI repair requires viable neurons, axon sprouting and long-tract re-growth across the trauma. Following SCI, the regeneration in the mammalian was extremely difficult and usually abortive due to the lack of support at the injury site [1]. Therefore, how to create a favourable environment for the transplanted therapeutic cells as well as neurons is critical for SCI repair.

Three major approaches have been used to promote axon regeneration: (1) Cell transplantation such as OECs [13]. (2) Addition of regeneration molecules including neurotrophic factors [18]. (3) Using biomaterial scaffolds made of natural [17] or synthetic material [9] to guide cell growth. However, none of the single approach was satisfactory in SCI repair and combined approaches such as poly (D,L-lactic acid) guidance scaffolds combined with brain-derived neurotrophic factor (BDNF) [15], cAMP pulsing Schwann cells [16] had been tested, and encouraging results were obtained in animal models. In the reported biomaterials, biodegradable materials were shown to have more advantages than non-biodegradable ones. As one biodegradable material, collagen is promising due to its abundant sources, good

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doi:10.1016/j.neulet.2006.02.085

plasticity, and biocompatibility [12]. Studies have also shown that the axon would be regenerated and its function would be partially restored after collagen was grafted parallel to the axis of the transected spinal cord [21].

Although the behaviors of OECs in 2-D culture has been widely documented, little is known about that of OECs in 3-D culture. The aim of this study was to reveal the behaviors of OECs on 3-D collagen scaffolds. We analyzed the phenotypes of OECs such as cell proliferation, apoptosis, morphology, and gene activities of some neurotrophic factors and the myelin proteins in both 2-D and 3-D culture. This study would allow us to better understand the effects of OECs or other related cells in 3-D culture environment and possibly lead to a better treatment by using these cells.

In this study, 3-D collagen scaffolds were obtained from Zhenghai Biotechnology Inc (YanTai, China). Scanning electron microscope (SEM) was used to visualize their surface feature. The structure of 3-D collagen scaffolds was shown in Fig. 1A. The average pore size was 20–100 μm in diameter. This would provide sufficient space for cells growing into the scaffold.

OECs were isolated from adult male Sprague–Dawley rats. The method was described in previous report with slightly modification [10]. All the experimental procedures were performed in accordance with the Principles of Laboratory Animal Care (NIH Publication, #85-23). Briefly, rats were anesthetized with 10% chloral hydrate (10 mg/kg, sigma) and decapitated. The two outer layers of the olfactory bulb were dissected, minced, and then trypsinized with 0.25% trypsin and 0.03% collagen enzyme IV for 15 min at 37 °C. After centrifuged twice (1000 rpm, 10 min), the pellet was suspended in medium DMEM/F12 (Gibco) with 10% fetal bovine serum (Gibco), 1% glutamine (Hyclone), 2% penicillin-streptomycin (Hyclone), and 1% gentamicin (Hyclone) and seeded into 60-mm cell culture dish (Corning, USA) with 5×10^6 cells and incubated for 24 h (37 °C and 5% CO_2). Most of the fibroblasts attached to the surface shortly. The supernatant was poured into second dishes and incubated for 48 h to allow for the attachment of astrocytes. Third supernatant was added into a poly-L-lysine (Sigma)-coated dish and into 3-D collagen scaffolds in 60-mm bacteria culture plastic dish, respectively. Only

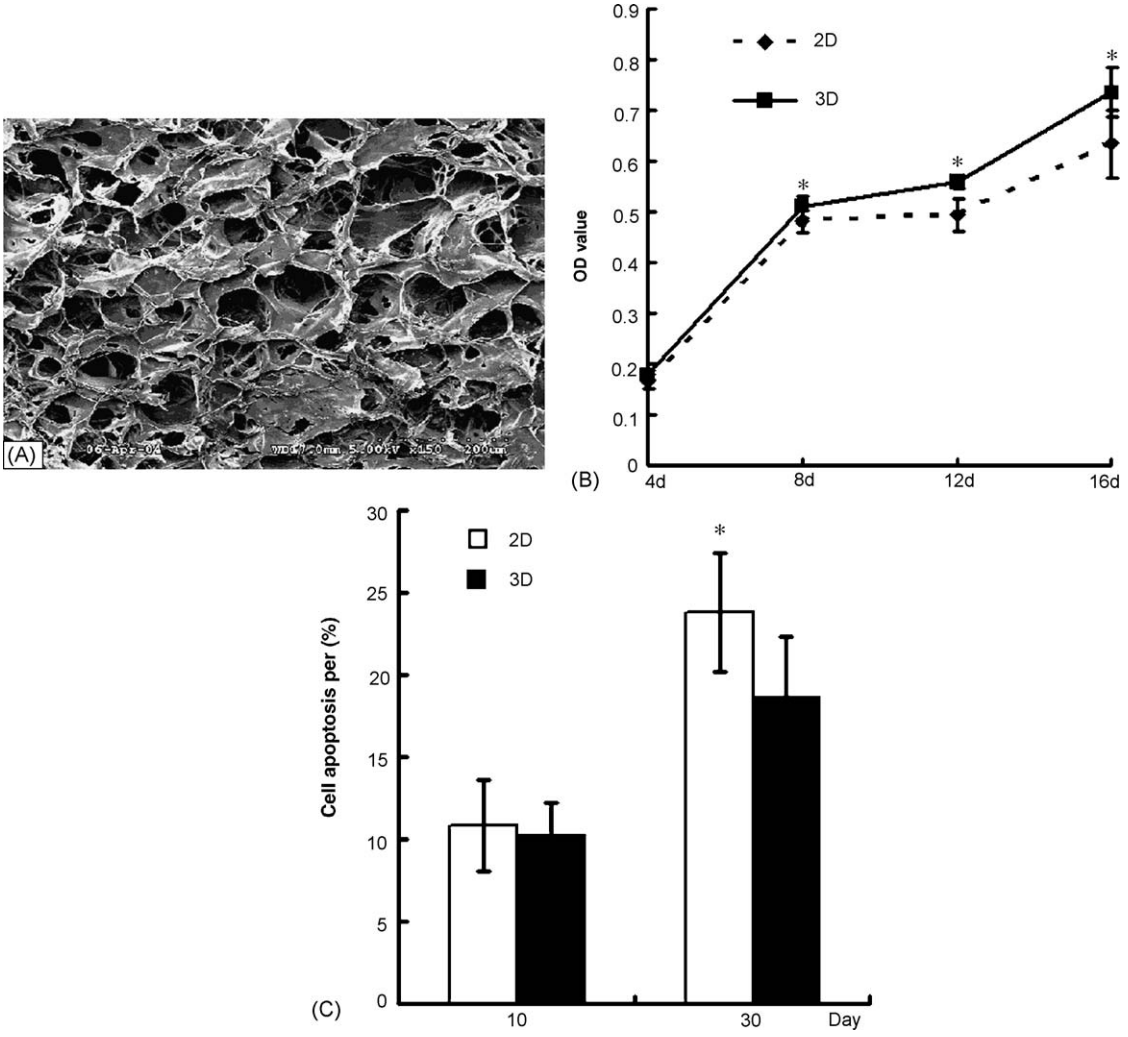

Fig. 1. Results of proliferation by MTT and cell apoptosis assay. (A) The structure of 3-D collagen scaffolds used in this work observed by SEM. (B) In vitro proliferation of adult rat OECs measured by MTT assay. The OD value of OECs on 3-D collagen scaffolds was higher than that in 2-D culture every time point. (C) Results of cell apoptosis assay. The number of apoptotic OECs on collagen scaffolds was lower than that in 2-D culture ($^*P < 0.05$).