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Research Report

The hetero-transplantation of human bone marrow stromal cells carried by hydrogel unexpectedly demonstrates a significant role in the functional recovery in the injured spinal cord of rats

Raynald[a], Yanbin Li[e], Hao Yu[c], Hua Huang[a], Muyao Guo[d], Rongrong Hua[b], Fenjun Jiang[b], Kaihua Zhang[a], Hailong Li[a], Fei Wang[f], Lusheng Li[c], FuZhai Cui[d], Yihua An[b,c,*]

[a]Beijing Neurosurgical Institute, Capital Medical University, Beijing 100050, China
[b]Department of Stem Cell Transplantation, The General Hospital of Chinese People's Armed Police Forces, Beijing 100039, China
[c]Department of Neurosurgery, Beijing Sanbo Brain Hospital, Capital Medical University, Beijing 100093, China
[d]Biomaterials Laboratory, Department of Materials Science and Engineering, TsingHua University, Beijing 100054, China
[e]Beijing Di Tan Hospital, Capital Medical University, Beijing 100050, China
[f]Department of Neurosurgery, Inner Mongolia Medical University Affiliated Hospital, Inner Mongolia 010050, China

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ABSTRACT

Spinal cord injury (SCI) often causes a disturbance in the microenvironment in the lesion site resulting in sudden loss of sensory and motor function. Transplantation of stem cells provides a promising strategy in the treatment of SCI. But limited growth and immunological incompatibility of the stem cells with the host limits the application of this strategy. In order to get better survival and integration with the host, we employed a hyaluronic acid (HA) based scaffold covalently modified by poly-L-Lysine (PLL) as a vehicle to deliver the human bone marrow stromal cells (BMSCs) to the injured spinal cord of rats. The BMSCs were chosen as an ideal candidate for its advantage of low expression of major histocompatibility complex II. The data unexpectedly showed that the hetero-transplanted cells survived well in the lesion site even at 8 weeks post injury. Both the immunofluorescent and the electrophysiological assay indicated better survival of the transplanted cells and improved axonal growth in SCI rats transplanted with BMSCs in HA-PLL in contrast to the groups without either BMSCs or the HA scaffold transplantation. These promotions

Abbreviations: BBB scale, the Basso Beattie Bresnahanlocomotor rating scale; BMSCs, bone marrow stromal cells; BrdU, bromodeoxyuridine; EDC, 1-ethyl-N,N-dimethy-laminopropyl carbodiimide; GFAP, glial fibrillary acidic protein; HA, hyaluronic acid; MEP, motor evoked potential; MHC-II, major histocompatibility complex antigen II; NeuN, neuronal nuclear antigen; NF, neurofilament; PLL, poly-L-lysine; SCI, spinal cord injury; SD rat, Spraque–Dawley rat; SEM, scanning electron microscope; TEM, transmission electron microscope

*Corresponding author at: Department of Stem Cell Transplantation, The General Hospital of Chinese People's Armed Police Forces, Beijing 100039, China.
E-mail addresses: raynald_liu@yahoo.com (Raynald), doctoran2010@hotmail.com (Y. An).

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may account for the functional recovery assessed by Basso–Beattie–Bresnahan (BBB) locomotor rating scale in the HA-PLL seeded with BMSCs group. These data suggests that hetero-transplantation of human BMSCs delivered by HA scaffold demonstrates a significant role in the functional recovery in the injured spinal cord of rats.

1. Introduction

Spinal cord injury (SCI) is generally characterized by the deficit in sensory and motor function caudal to the level of injury due to the destruction of neurons and the failure of axonal regeneration in the deteriorated microenvironment in the lesion site. In this microenvironment, massive inflammation, edema, demyelination, cell death, vascular destruction, formation of glial scar and cavity are found (Schwab, 1998; Tator and Fehlings, 1991). As a promising treatment strategy for SCI, cell therapy is employed to replace the injured cells and secrete neurotrophic factors (Mothe and Tator, 2012; Tetzlaff et al., 2011). Several cell based therapeutic studies display evident axonal growth and improvement in functional recovery in rodent SCI models (Johnson et al., 2010; Ritfeld et al., 2012). And the effect is significantly influenced by the immunological incompatibility with the host and inefficient stem cell growth *in vivo* (Wakayama et al., 2001; Xu et al., 2005).

On account of low expression of major compatibility complex antigens II (MHC-II), rapid propagation and easy accessibility, BMSCs are regarded as an ideal candidate cell type for transplantation (Nakano et al., 2010; Ringden and Le Blanc, 2005). There are mainly two mechanisms by which BMSCs perform their function in the injured neural tissue. One is that the transplanted BMSCs can differentiate into different cell types such as neurons, oligodendrocytes and astrocytes which help to replace the tissue lost (Nandoe Tewarie et al., 2009; Xu et al., 2011). The other is that the transplanted cells can release a series of factors that may provide trophic support for the injured tissue in the lesion site (Novikova et al., 2011; Ritfeld et al., 2012). Thus, such a cell type of low antigenicity can be expected to play an important role in the restoration of the injured tissue. And our study unexpectedly showed that the hetero-transplanted human BMSCs survived well in the lesion site even at 8 weeks after injury.

As is mentioned above, the microenvironment in the lesion site is neurotoxic. In an attempt to achieve a neuroprotective microenvironment to improve the survival of transplanted cells, several groups employed biomaterials such as solid scaffolds to deliver the stem cells and showed improved viability of transplanted cells in rat models of SCI (Guo et al., 2012; Kim et al., 2011; Nomura et al., 2006). The promotion of cell survival can be attributed to the hyaluronic acid (HA) in the HA-PLL scaffold which is a key component of the extracellular matrix (ECM) (Ren et al., 2009). And the interaction between HA and cells through CD44and RHAMM receptors exerts influences on cell survival and migration (Aruffo et al., 1990; Casini et al., 2010; Martino and Pluchino, 2006; Turley, 1992).

In this study, we employed a HA based scaffold to deliver the human BMSCs to the lesion site of SCI rats. Prior to use, we examined whether the BMSCs survived well in this scaffold. We then investigated the survival, differentiation of the hetero-transplanted BMSCs and the axon regeneration using multiple technologies. Finally, Basso–Beattie–Bresnahan (BBB) locomotors rating scale was employed to test the functional recovery of SCI rats.

2. Results

2.1. *Characterization of human BMSCs and the chemical structure of HA-PLL*

BMSCs were isolated from the posterior superior iliac spine of the donor patient. And cells were collected after adherence and passaged for five times for FCM analysis which aimed to detect the cell purity prior to use. The phenotypes of BMSCs used in our study were positive for CD44 (97.97%), CD73 (99.99%), CD90 (99.97%) and CD105 (98.94%), and negative for CD11b (0.26%), CD19 (1.62%), CD34 (1.42%) and CD45 (1.08%; Fig. 1A). This is consistent with the previous study (Vaquero and Zurita, 2009). Then the cells were used for transplantation. The HA based scaffold was synthesized in the way as shown (Fig. 1B).

2.2. *In vitro survival of human BMSCs in HA-PLL*

Scanning electron microscope (SEM) analysis showed the multi-porous three-dimension ultra-structure of HA-PLL (Fig. 2A). This structure was designed to facilitate the axonal outgrowth along the porous structure in the injured tissue. In order to observe the survival of the seeded cells, we transplanted human BMSCs into the HA-PLL hydrogels in the form of cell suspension. When cultured for 3 days, both SEM and optical microscope data indicated that the human BMSCs survived well on the surface of HA-PLL (Fig. 2B–F). In addition to this, cell viability assay showed that there was no statistical difference in viability of BMSCs between in media only and in media containing HA-PLL (Fig. 2G). This indicates that HA-PLL is not toxic to the BMSCs *in vitro*. Then the further *in vivo* experiments were performed.