Biomaterials 34 (2013) 5937–5946

Contents lists available at SciVerse ScienceDirect

Biomaterials

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

Transplantation of human mesenchymal stem cells loaded on collagen scaffolds for the treatment of traumatic brain injury in rats

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ARTICLE INFO

Article history:
Received 27 February 2013
Accepted 23 April 2013
Available online 9 May 2013

Keywords:
Brain trauma
Nuclear imaging
Stem cells
Biomaterials
Differentiation

ABSTRACT

Studies have suggested that mesenchymal stem cells (MSCs) have therapeutic effects following traumatic brain injury (TBI). However, cell distribution and survival rate are two major barriers to their success as therapeutic treatment. The improvement of cell therapy using collagen delivery matrices had been reported. However, we know very little about the mechanisms. We labeled human bone marrow-derived mesenchymal stem cells (hMSCs) with a positron emission tomography (PET) tracer, 18F-fluoro-2-deoxy-D-glucose (FDG). hMSCs were transplanted with or without collagen scaffolds into rats with experimental TBI and the whole-body nuclear images were compared. Collagen scaffolds increased the retention of hBMSC in the lesion site and limited its distribution at the transplanted region. Significantly more hMSCs were detected in the brain when transplanted with collagen scaffolds. The results showed collagen scaffolds also efficiently improved cell survival and neurite outgrowth *in vivo*, resulting in better neural functional recovery. In addition, brain metabolism also improved in the collagen scaffold implanted group, as evaluated by PET. We speculated that collagen scaffolds would improve early engraftment and support the survival of grafted cells post-transplantation.

1. Introduction

Traumatic brain injury (TBI) is a serious public health problem worldwide. It causes death and disability, such as cognitive deficits, dysphasia, and hemiplegia [1,2]. After TBI, especially the old TBI, loss of cerebral parenchyma is a prominent obstacle of functional recovery. At present, there is no effective clinical treatment for repairing TBI-related loss of cerebral parenchyma. Studies showed that stem/progenitor cell transplantation holds great promise for treating TBI [3]. Mesenchymal stem cells (MSCs), administrated by either direct intracerebral injection or systemic injection after TBI, have been successful in reducing loss of brain tissues and improving neurological functional recovery in experimental central nervous system (CNS) injury animal models [4–6]. Human bone

Abbreviations: MSCs, mesenchymal stem cells; TBI, traumatic brain injury; hMSCs, human bone marrow-derived mesenchymal stem cells; ESCs, embryonic stem cells; FDG, fluorodeoxyglucose; PET, positron emission tomography; ROI, region of interest; DAPI, 6-diamidino-2-phenyl-indole.

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http://dx.doi.org/10.1016/j.biomaterials.2013.04.047

marrow-derived MSCs (hMSCs) are less immunogenic, less tumorigenic, and have fewer ethical problems than embryonic stem cells (ESCs) [7,8].

The curative effects of stem cell treatment depend on the localization of cells grafted in the target tissue [9]. However, stem cell therapy is hindered by insufficient concentration of cells within the target tissue and the low survival rate of cells grafted [10,11]. Firstly, due to its rapid diffusion to cerebrospinal fluid, transplanted cells could be removed quickly from the target tissue, usually within hours or a few days [10]. Insufficient cell numbers and inadequate cellular interactions are adverse factors of providing therapeutic effect [12]. In addition, associating with the delivery of cells to non-targeted tissues, there is a risk of unwanted side effects [13]. The other major obstacle to the curative effects of stem cell treatment is the low survival rate of grafted cells, which might be caused by the mechanical damage, acute inflammation, immunological rejection, or the lack trophic factors [11,14]. Thus, for successful transplantation, development of a suitable environment to improve stem/progenitor cell engraftment and survival is crucial. Collagen is the most abundant and accessible protein in animals [15]. Previous work, including our own, has demonstrated that tissue-engineered collagen-based scaffolds can provide a suitable microenvironment to support cell attachment and proliferation [16–18]. In this study, we optimized a porous, biodegradable gelatinous collagen scaffold. Using the TBI rat model, hMSCs were injected with or without collagen scaffolds, and we proposed that the collagen can provide local physical retention, mimic the extracellular scaffolding, provide physical cues for cell spreading and orientation, and support the survival of grafted stem cells.

Past evaluation of the ability of scaffolds to improve cell engraftment within the brain has so far been limited primarily to regional histological or immunohistological analyses at a single time point [19]. After implantation, the dynamic assessment of cell migration help us to find out the *in vivo* state of cells [20]. Therefore, in this study, noninvasive *in vivo* imaging was used to provide quantitative information about the biodistribution and proliferation of the engrafted stem cells in the region of interest. The therapeutic effects were estimated by ethology score, molecular imaging technology, and immunohistochemistry.

2. Materials and methods

2.1. Preparation and labeling of hMSCs

The hMSCs were isolated from bone marrow aspirate from a healthy donor who gave informed consent. The isolation and culture were conducted in the Center of Excellence in Tissue Engineering (Institute of Basic Medical Sciences and School of Basic Medicine). The hMSCs were used in accordance with the procedures approved by the Human Experimentation and Ethics Committee. Cell culture was performed as previously described [21]. The hMSCs were collected, counted, and analyzed by flow cytometry for phycoerythrin anti-human phycoerythrin-CD29, CD105, CD44, Flk-1, CD34, CD44, and HLA-DR expression (Becton Dickinson, Franklin Lakes, NJ) as previously described [22].

The 18F-fluorodeoxyglucose (FDG) labeling was carried out by the Department of Nuclear Medicine (Peking Union Medical College Hospital, Beijing, China) following procedures as described previously [23]. For each operation, approximately 1×10^7 hMSCs were incubated with 25 MBq of FDG in 1 mL of medium at 37 °C for 40 min. To improve labeling efficiency, insulin (0.1 U/mL) and heparin (10 U/mL) were added. After incubation, cells were rinsed twice to remove unbound radioactive material. The radioactivities of both the cells and the supernatant were measured using a dose calibrator (Capintec, Syncor, USA). Cell-labeling efficiency was expressed as a percentage, which was calculated using the formula: (activity in cells/total activity used in the incubation) × 100%. Cells were resuspended in 1 mL of phosphate-buffered saline (PBS) prior to injection. These labeling conditions were chosen according to preliminary *in vitro* assays (data not shown).

2.2. Stability and viability of labeled cells

To assess the stability of labeled cells, they were rinsed, centrifuged and resuspended in 2 mL PBS, and incubated at 37 °C. Efflux of FDG was determined by measuring the radioactivity of the medium collected at 5, 30, 180, 360, and 480 min. The retention of 18F-FDG within hMSCs was calculated by measuring the radioactivity of the cell pellet. Percentage of FDG retention was expressed as (the radioactivity in cell lysate)/(the sum of radioactivities of the cell lysate and the supernatant) × 100%.

The effects of labeling on cell viability were determined by the Cell Counting Kit-8 (CCK8; Dojindo Laboratories, Kumamoto, Japan) according to manufacturer's instructions [24]. Cells were seeded in 96-well plates at 1.0×10^4 cells/well. Briefly, 10 μL of CCK8 solution was added to the culture medium. The plates were incubated for additional 3 h. The absorbance at 450 nm was measured by microplate reader at 3, 24, and 48 h after labeling.

2.3. Preparation of collagen scaffold and hMSCs-seeded scaffold

The porous, gelatinous collagen scaffolds (collagen type I) were obtained from the Key Laboratory of Molecular Developmental Biology (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, PR China).

We kept the transplant components (i.e., hMSCs, media, and collagen scaffolds) on ice and mixed them immediately prior to injection.

2.4. Surgical procedure and injection of hMSCs

All experiments conformed to the guidelines issued by the committee on animal research of Peking Union Medical College Hospital and approved by the institutional ethics committee.

Adult male Sprague–Dawley rats (230–250 g, $n = 42$) were purchased from the Center for Experimental Animal Research of the Chinese Academy of Medical Science, Beijing, China. Traumatic brain injury (TBI) was induced using a controlled cortical impact (CCI) model [25]. In brief, each rat was anesthetized with 10% chloral hydrate (400 mg/kg) then placed in a stereotactic frame. One 5-mm diameter craniotomy was performed over the left parietal cortex (midway between bregma and lambda sutures and adjacent to the central suture). The left cortex was impacted with a pneumatic piston containing a 5-mm diameter tip at a rate of 4 m/s and 2.5 mm of compression. The collagen scaffold was transplanted into the core of the lesion 7 days after CCI injury.

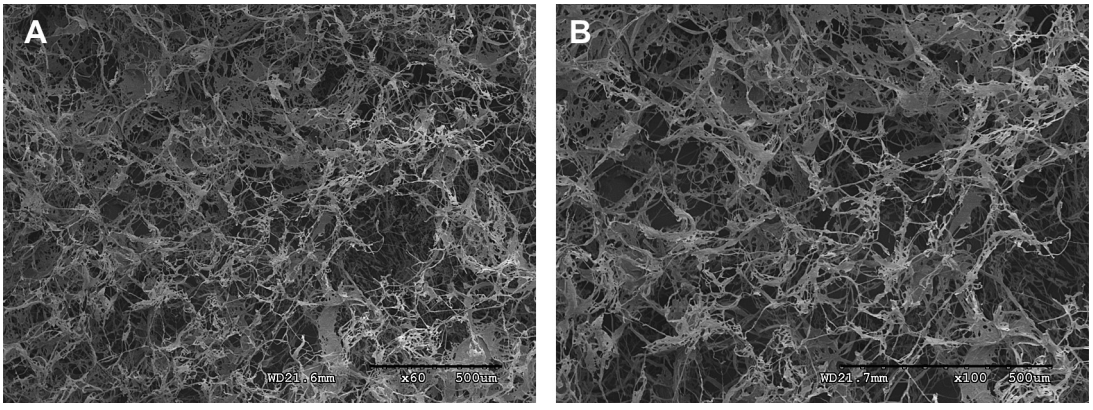

Fig. 1. SEM of the injectable collagen gel displayed fibrillar networks of collagen fibers suitable for cell attachment. (A) ×60 objective, (B) ×100 objective. Scale bar: 500 μm.