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Transplantation of RADA16-BDNF peptide scaffold with human umbilical cord mesenchymal stem cells forced with CXCR4 and activated astrocytes for repair of traumatic brain injury

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ABSTRACT

Due to the poor self-regeneration of brain tissue, stem cell transplantation therapy is purported to enable the replacement of lost neurons after traumatic brain injury (TBI). The main challenge of brain regeneration is whether the transplanted cells can survive and carry out neuronal functions in the lesion area. The brain is a complex neuronal network consisting of various types of cells that significantly influence on each other, and the survival of the implanted stem cells in brain is critically influenced by the surrounding cells. Although stem cell-based therapy is developing rapidly, most previous studies just focus on apply single type of stem cells as cell source. Here, we found that co-culturing human umbilical cord mesenchymal stem cells (hUC-MSCs) directly with the activated astrocytes benefited to the proliferation and neuron differentiation of hUC-MSCs in vitro. In this study, hUC-MSCs and the activated astrocytes were seeded in RADA16-BDNF peptide scaffold (R-B-SPH scaffold), a specifical self-assembling peptide hydrogel, in which the environment promoted the differentiation of typical neuron-like cells with neurites extending in three-dimensional directions. Moreover, the results showed co-culture of hUC-MSCs and activated astrocytes promoted more BDNF secretion which may benefit to both neural differentiation of ectogenic hUC-MSCs and endogenic neurogenesis. In order to promote migration of the transplanted hUC-MSCs to the host brain, the hUC-MSCs were forced with CXC chemokine receptor 4 (CXCR4). We found that the moderate-sized lesion cavity, but not the large cavity caused by TBI was repaired via the transplantation of hUC-MSCs^{CXCR4} and activated astrocytes embedded in R-B-SPH scaffolds. The functional neural repair for TBI demonstrated in this study is mainly due to the transplantation system of double cells, hUC-MSCs and activated astrocytes. We believe that this novel cell transplantation system offers a promising treatment option for cell replacement therapy for TBI.

Statement of Significance

In this reach, we specifically linked RGIDKRHWNSQ, a functional peptide derived from BDNF, to the C-terminal of RADARADARADARADA (RADA16) to structure a functional self-assembling peptide hydrogel scaffold, RADA16-BDNF (R-B-SPH scaffold) for the better transplantation of the double cell unit. Also, the novel scaffold was used as cell-carrier for transplantation double cell unit (hUC-MSCs/astrocyte) for

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treating traumatic brain injury. The results of this study showing that R-B-SPH scaffold was pliancy and flexibility to fit the brain lesion cavity and promotes the outgrowth of axons and dendrites of the neurons derived from hUC-MSCs in vitro and in vivo, indicating the 3D R-B-SPH scaffold provided a suitable microenvironment for hUC-MSC survival, proliferation and differentiation. Also, our results showing the double-cells transplantation system (hUC-MSCs/astrocyte) may be a novel cell-based therapeutic strategy for neuroregeneration after TBI with potential value for clinical application.

1. Introduction

Traumatic brain injury (TBI) presently remains a challenging clinical problem. Both the immediate destruction caused by TBI as well as secondary injuries due to the delayed release of inflammatory mediators and ischaemia may cause continuous neuronal injury, resulting in brain tissue defects and severe disability [1]. Moreover, neurons show little ability to self-repair, and no therapy to efficiently reverse neuronal injury has been made available until now [2]. Due to the limited number of endogenous neural stem cells (NSCs), the lack of an appropriate niche for cell adhesion and the absence of effective guiding semaphores for neuronal differentiation, the brain's ability to repair itself is insufficient, especially in the case of cortical colobomas caused by TBI [3,4]. Therefore, the transplantation of ectogenic stem cells is required to overcome the insufficiency of endogenic stem cells in the central nervous system (CNS) [5].

Recently, investigators increasingly believe that augmentation with exogenous stem cells other than the endogenous NSCs of the brain may enhance meaningful recovery in the CNS [6–8]. Current data suggest that mesenchymal stem cells (MSCs) may provide therapeutic benefits in animal models of various neurological disorders, including stroke, Parkinson's disease and TBI [9,10]. Human umbilical cord mesenchymal stem cells (hUC-MSCs) isolated from human umbilical cord (hUC) have shown clinical therapeutic potential because of their characteristics, which include their extensive sourcing, low immunogenicity, potential for differentiation, ease of acquisition and clinical application prospects [11,12]. In fact, hUC-MSCs have been shown to possess the potential to differentiate into neurons, which has inspired their introduction into the field of brain repair. Therefore, in this study, we chose hUC-MSCs as the seed cells for cell transplant therapy after TBI.

Numerous studies investigating cell transplantation for CNS regeneration have been conducted in recent decades. Although different types of seed cells have been developed for transplantation recently, most previous interventions just focus on the delivery of single stem cells as the only transplanted cell source [13–15]. However, as we all know, the brain is a complex neuronal network consisting of various types of cells, including neurons, astrocytes, oligodendrocytes, and microglia. Each type of cell in the brain has significant influences on the other brain cells in terms of support, neurotrophic effects and signalling. Therefore, the surrounding niche, including the sustentacular cells in the CNS, is critically important for the survival and neuronal regeneration of transplanted stem cells. Astrocytes are the most abundant glial cells in the CNS and perform many important functions, including the biochemical support of cells in the CNS, the provision of nutrients to the nervous tissue, glial transmission and signalling [16,17]. Recent research has also demonstrated that sustentacular cells, such as astrocytes, are necessary to provide a niche for the proliferation and differentiation of NSCs [18]. Although the reactive astrocytes in lesion brain were traditionally assumed to impede neuronal regeneration because of gliosis, more and more evidences have indicated they do participate in functional recovery of brain injuries [19–21]. For example, they release neurotrophic factors, surface recognition molecules and cytokines, providing energy substratum in supporting neuronal survival, axonal outgrowth and synaptic reorganization. They can also prevent neurons from oxidant stress and promote neurogenesis. Therefore, we reasoned that the use of a double-cell transplantation system rather than single cells might promote the efficiency of hUC-MSCs replacement therapy after TBI. In this study, we first try to employ activated astrocytes as sustentacular cells direct combined with hUC-MSCs co-seeded in a biomaterial scaffold for the cell transplantation.

Some investigators have found that cell injection alone is inadequate for TBI that involves cortical cavities, which must be filled with a physical support that permits the engraftment of transplanted cells and the restoration of the cytoarchitecture [22,23]. Moreover, recent research has shown that the specialized microenvironment provided by biomaterial scaffolds is critical for the survival, migration and functionalization of transplanted cells [11,24]. The concept of self-assembly has been applied to the design and development of new biomaterials with prominent application value for tissue engineering [25]. However self-assembling peptide scaffolds seldom applied in TBI to repair brain defects. RADA16 (RADARADARADARADA) is a self-assembling, biocompatible peptide that exhibits controlled biodegradation, no cytotoxicity or immunogenicity, and ease of production and modification [26]. In this study, we used the hydrogel RADA16 to create a biomaterial scaffold for cell transplantation. Because BDNF has been shown t o promote neurotrophy, cell proliferation, neuronal differentiation and neurite outgrowth [27,28], we specifically linked RGIDKRHWNSQ [29], a peptide derived from BDNF, to the C-terminal of RADA16 to structure the scaffold. Therefore, in this study, we first designed the RADA16-BDNF peptide scaffold such that it could not only flatten the injured brain cavity and reduce the surrounding reactive gliosis but could also provide neurotrophic factors.

Stromal cell-derived factor-1 (SDF-1), a member of the CXC chemokine subfamily, mediates the homing of stem cells associated with injury repair by binding to CXCR4, the primary physiological receptor for SDF-1 on targeted cells [30]. SDF-1 gene expression is regulated by the transcription factor hypoxia-inducible factor-1 (HIF-1) in vivo [31]. However, it has been reported that the level of CXCR4 expression on the membrane of hUC-MSCs is low, and that it is reduced even further in proliferating cells, thereby limiting the migratory ability of the cells [32]. Therefore, in this study, we first specifically transferred CXCR4 to hUC-MSCs to improve the migratory ability of the transplanted hUC-MSCs^{CXCR4}.

In this study, we specifically chose hUC-MSCs and activated astrocytes as the seed cells and performed double-cell unit transplants to promote the vitality and neuronal differentiation of hUC-MSCs during regeneration after TBI. The aim of this work was to provide a novel biomaterial scaffold containing encapsulated the fresh double cell unit (hUC-MSCs^{CXCR4}/activated astrocytes) for use as a future cell replacement therapy for TBI involving cortical coloboma.