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Original research

A comparison between nucleus pulposus-derived stem cell transplantation and nucleus pulposus cell transplantation for the treatment of intervertebral disc degeneration in a rabbit model

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HIGHLIGHTS

- Both nucleus pulposus-derived stem cells (NPSCs) and nucleus pulposus cells (NPCs) expressed stem cells surface markers.
- NPSCs harvested by differential adhesion method had higher mesenchymal stem cells surface markers than NPCs.
- NPSCs transplantation showed better regenerative effects for intervertebral disc degeneration compared with NPCs.

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ABSTRACT

Introduction: In recent years, nucleus pulposus cell (NPC) transplantation has been used to treat intervertebral disc degeneration (IDD); however, the degenerative nature of NPCs influences its effectiveness. Nucleus pulposus-derived stem cells (NPSCs), which are self-renewing, have high expansion potential and can adapt to the intervertebral disc (IVD) microenvironment and may have a better regenerative capacity, which is favourable for treating IDD. The aim of this study was to compare the effectiveness of transplantation with NPSCs and NPCs on the regeneration of the IVD in rabbit models.

Methods: NPSCs and NPCs were isolated from human degenerate nucleus pulposus tissue by differential adhesion method, and stem cell surface markers were detected by flow cytometry. Degenerative discs in rabbits were randomly distributed into three groups: NPSCs, NPCs and vehicle control group; the normal discs served as the normal control group. Cells of the P3 generation were prepared for transplantation. About 20 μl of cell suspension (NPSCs or NPCs) or DMEM was injected into corresponding discs, while the normal discs were left untreated. After 8 weeks, disc height was evaluated using X-ray, water content was evaluated by MRI, and gene and protein expression levels of collagen II and aggrecan in the nucleus were determined by real-time PCR and ELISA.

Results: NPCs and NPSCs from the P3 generation were polygonal and spindle-shaped, respectively. Both NPSCs and NPCs strongly expressed surface markers CD73, CD90, and CD105 and weakly expressed CD34 and CD45. The relative rates of expression of CD73, CD90, and CD105 were higher in NPSCs than in NPCs. After 8 weeks, X-ray results showed no significant difference in disc height index among the groups ($p > 0.05$). MRI revealed that the intensity of the nucleus pulposus signal was increased in NPSCs ($p < 0.05$). The results from PCR and ELISA demonstrated that NPSCs promoted gene and protein expression of aggrecan instead of collagen II ($p < 0.05$).

Conclusion: Compared to NPCs, NPSCs harvested by differential adhesion method displayed a higher positive rate of stem cell surface markers and showed superior regenerative effectiveness for treating IDD in rabbit models. Therefore, NPSCs are potential candidates for cell therapy for the regeneration of the IVD.

1. Introduction

Low back pain is one of the most common health conditions, associated with high medical costs and patient morbidity [1].

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Intervertebral disc degeneration (IDD) is a major cause of low back pain [2]. The normal intervertebral disc (IVD) has three distinct components: the central hyperhydrated nucleus pulposus (NP), the outer annulus fibrosus, and the upper and lower cartilaginous endplates [3]. Although the causes of IDD degeneration are still largely unknown, a decrease in the function and number of nucleus pulposus cells (NPCs) is an initial trigger of IDD [4,5]. Various operative methods such as spinal fusion and artificial disc replacement have been used to treat degenerative disc diseases and have shown satisfactory results in alleviating pain. They are, however, not devoid of complications, including accelerated degeneration of the levels adjacent to the fusion or the prosthetic disc's migration, extrusion or failure [6,7]. Most importantly, these treatments aim to alleviate patients' symptoms, but do not target the underlying disease itself. None of these treatments aims to restore the biological function of the disc nor slow down or reverse IDD [8,9]. Recently, cell transplantation has become one of the major biological procedures used to treat IDD [8–10].

Due to their self-renewing ability, high expansive potential in culture, and capacity for multilineage differentiation, stem cells are a more attractive cell source for the treatment of IDD [10]. Many types of stem cells have been used in cell transplantation for the treatment of IDD, such as bone marrow-derived stem cells (BMSCs) [11], adipose-derived stem cells (ADMSCs) [12], human umbilical tissue-derived cells [13], and synovium-derived stem cells [14]. However, the stem cells must survive and function in the harsh IVD microenvironment for successful cell therapy. The IVD microenvironment is characterised by high osmolarity, limited nutrition, acidic pH, and low oxygen tension [9,15,16]. Unfortunately, some studies have reported that stem cells lack viability, proliferation, and matrix biosynthesis under IVD microenvironment, as it negatively influences the biological and metabolic vitality of stem cells and impairs their repair potential [17,18]. Therefore, it is necessary to find other cell sources for cell therapy for IDD.

Transplantation of NPCs has become one of the major techniques used experimentally to prevent IDD. However, NP tissues have low levels of cellular and proliferative activity [19]. The regenerative capacity of autologous disc cells harvested from herniated discs was questioned in an in vitro study, where the cells lost their differentiation potential and ability to synthesize aggrecan and collagen type II [20]. Moreover, the application of NPCs is limited because phenotypic changes displayed in the degenerated IVD cause NPCs to produce less extracellular matrix [21].

In 2007, Risbud et al. [22] provided evidence for the existence of endogenous progenitor cells in human NP tissue and demonstrated their capacity for multilineage differentiation in vitro. Recently, many researchers have demonstrated the existence of NP-derived stem cells (NPSCs) among various species [23,24] such as rat, mini pig, rabbit, and canine. Blanco et al. [25] demonstrated that NPSCs isolated from human degenerate NP tissue possess a capacity for chondrogenic differentiation similar to that of BMSCs. Han et al. [26] revealed that NPSCs were more adaptable to the harsh IVD microenvironment than ADMSCs were. Tao et al. [27] harvested NPSCs and NPCs through the method of differential adhesion, Tao et al. [27] harvested NPSCs and NPCs through differential adhesion and found that NPSCs and NPCs expanded from adherent primary cells within 1 day and 3 days, respectively.

In this study, we harvested NPSCs and NPCs from human degenerated nucleus pulposus tissue by differential adhesion. First, we aimed to study the differential expression of mesenchymal stem cell (MSC) surface markers in NPSCs and NPCs and second, to investigate the regenerative effects of NPSCs and NPCs in rabbit models of IDD.

2. Materials and methods

The research ethical committee of our hospital approved this study, and this paper was written in accordance with the Animal Research: Reporting In Vivo Experiments (ARRIVE) statement [28].

2.1. Isolation of NP tissue-derived cells

All the tissue samples were obtained from patients with moderately degenerative lumbar intervertebral disc while undergoing vertebral body fusion. NP tissue was detached from surrounding tissues such as vessels, annulus fibrosus, and ligaments. Each NP sample was cut into half and assigned for isolation as NPCs or NPSCs.

Isolation of NPCs was performed by enzymatic digestion. Tissue samples were washed three times with PBS and minced into 1-mm^3 pieces. Collagenase II solution at a concentration of 0.2% (m/v) was added, and the mixture was left to digest at 37 °C for 6 h. The mixture was filtered through a 200-mesh sieve and re-suspended in 10% FBS and high-glucose DMEM. Cells suspensions were then seeded in 6-well plates and cultured at 37 °C in a humidified atmosphere containing 5% CO_2. The complete medium was changed every 3 days. Cells harvested using this method were labelled NPCs. After three passages, NPCs were observed under the inverted microscope, and NPCs suspensions were prepared for analysis by flow cytometry and intradiscal implantation.

To isolate NPSCs, differential adhesion was performed as previously reported [27]. The cell extraction process was similar to the method used for NPC isolation. NP tissue samples were washed with PBS, minced into pieces, and digested with collagenase II solution at 37 °C for 6 h. The mixture was filtered through a 200-mesh sieve and re-suspended in 10% FBS and high-glucose DMEM. Cells suspensions were seeded in 6-well plates and cultured at 37 °C in a humidified atmosphere containing 5% CO_2. After 24 h, culture media with non-adherent cells were discarded and adherent cells were supplemented with fresh medium. The culture medium was renewed every 3 days. After three passages, NPSCs were prepared for identification by flow cytometry and for the animal experiments.

2.2. Flow cytometry assay

Flow cytometry assay was used to identify the expression of specific surface markers in the NPSCs and NPCs. In accordance with the minimal criteria for defining MSCs proposed by the International Society for Cellular Therapy (ISCT), the expression of surface markers like CD73, CD90, CD105, CD34, and CD45 was evaluated in this study [29]. About 1×10^6 cells were re-suspended with PBS (containing 5% FBS) to produce a single cell solution. NPSCs and NPCs were incubated separately with PE-tagged CD73 and CD105, FITC-tagged CD90, and APC-tagged CD34 and CD45 at room temperature. Finally, labelled cells were washed three times with PBS and surface marker expression was detected using flow cytometry.

2.3. Animal surgery

For induction of IDD, 24 male New Zealand rabbits (weight: approximately 2.5 kg; age: 6 months) were used. All animals were subjected to surgery and throughout the procedures they were housed in cages. The rabbits were monitored daily during the entire experimental procedure by an accredited veterinarian, trained in laboratory animal science. Lumbar discs 3–6 were designated the target discs for induction of degeneration and lumbar discs 6–7 were designated as the normal group. The surgical process was conducted as described by Masuda et al. [30]. After being