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# Transplanted neural-like cells improve memory and Alzheimer-like pathology in a rat model

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**Abstract**

*Background aims.* Degeneration of the central nerve system, particularly in Alzheimer's disease, is a burden on society, and despite years of research, there is no effective treatment. Cell therapy appears to be an option that is of growing interest in neural studies. The main aim of this study was to investigate the histological and physiological effects of transplantation the neuron-like cell (NLC)-derived mouse embryonic stem cells (mESCs) on the repair of brain lesions in an Alzheimer's animal model (AM) in rats. *Methods.* Behavioral experiments were conducted in the light hours in a Y-shaped maze device. Animals were randomly divided into five groups, with seven rats per group. The nucleus basalis of Meynert (NBM) was destroyed bilaterally with an electrical lesion (0.5 mA for 3 s). One week after the bilateral lesion of the NBM, the differentiated NLCs (0.1 mL) were injected with stereotaxic surgery using a Hamilton syringe at NBM coordinates, and behavioral and histological tests were performed by the Y-maze task and hematoxylin and eosin staining after five weeks of the lesion. Also, differentiated cells detected by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis and fluorescent immunostaining. *Results.* The expression of neuronal markers including Nestin, Map2, NF-H, Tuj-1, GFAP and Olig-2 was surveyed by using the immunocytochemistry and qRT-PCR methods, and the results confirmed that the genes in question were expressed significantly more compared than the control sample. Five weeks after the cell transplantation in the AM, morphological and physiological investigation during the determination period confirmed improved disease state in the tested models. *Conclusions.* It should be noted that by improving the neuronal connectivity in AM rat brains, the transplanted NLCs rescue Alzheimer's cognition. This research has presented some preclinical evidence that showed NLCs transplantation can be used for AM treatment.

**Key Words:** *Alzheimer's animal model, differentiation, embryonic stem cells, neuron cells, physiological and histological*

## Introduction

One of the most prominent phenomena of this century is population aging, and a number of common diseases increase in the elderly population, including Alzheimer's disease (AD). The prevalence of this disease increases exponentially with age [1,2]. AD is a type of progressive brain impairment that gradually impairs memory, learning, reasoning, and the ability to do daily activities. It virtually destroys neurons and disrupts the relationship between the cells, and through the precipitation of harmful chemical compounds, the transmission of nerve messages becomes difficult [3,4]. The symptoms of this disease can also be due to the defective structure of the new hippocampus neurons in dentate gyrus, which affects the mental state of learning and memory [5]. This degeneration of the central nerve system (CNS) is a burden on society, and despite years of research, there is no effective treatment. However, cell therapy appears to be an option that is of growing interest in neural studies. Today, with the advancement of basic science and tissue engineering, cell therapy offers new treatment for many diseases, including neural disorders [6,7]. When cell therapy becomes widespread, a healthier life and increased longevity may result. Hence, several countries have invested resources in this area and encouraged patients to undergo such treatment [8,9].

Unlike most body tissues, the nervous system has limited restoration capacity. Despite the presence of neural stem cells, the ability to produce new and active neurons in response to damage is also limited, and so an examination of other cellular resources that can replace the damaged nerve cells is necessary [10]. In this regard, researchers have used various cellular resources, such as olfactory epithelial cells [11],

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mesenchymal stromal cells [12], neural precursors and embryonic stem cells (ESCs) thus far. ESCs are considered suitable candidates in terms of their special properties for cell therapy [13,14]. ESCs derived from the inner cell mass in blastocyst are being produced. These cells have pluripotency and are capable of self-renewal and differentiation into other cells, including ectodermal, mesodermal and endodermal cells [14]. One of the important characteristics of these cells compared with others is their rapid proliferation. ESCs have a normal karyotype and preserve pluripotentiality during sequential passages. Thus, their ability to proliferate and their high differentiation offers the possibility of producing countless nerve cells, and their use in cell therapy has been demonstrated. Previous studies have shown that it is possible to differentiate these stem cells into neural cells *in vitro* [15,16].

Researchers have begun to investigate various fatty acids, especially the unsaturated fatty acids, which play important roles in human body health. The CNS is a strategic system that contains a high density of the unsaturated fatty acids. Therefore, it has been suggested that fatty acids have a considerable role in neural hemostasis and its development. Conjugated linolenic acid (CLA) is a member of the linolenic acid family that is used as a dietary supplement and has various benefits. Although CLA was noted more than as anticancer, it has some other important biological activities [17,18].

One of the goals of cellular research is to track the fate of cells. Most cell markers are fluorescent or can create a process that produces a visible color reaction. Fluorescent markers are stimulated with light-emitting visible wavelengths, and they reflect light at a higher wavelength. One of the cell trackers in live cells is DiI dye, which is a lipophilic and fluorescent dye and diffuses laterally to stain the entire cell. This dye is not toxic and, as has been reported, remains in cells for long periods. It is also seen in fixed tissue. This dye has the same characteristics as rhodamine, and under green light, it will emit a fluorescent red light [19,20].

The main aim of this study was to investigate the histological and physiological effects of transplantation of mouse ESCs differentiated from the neuron-like cells on the repair of brain lesions in an AD animal model in rats. Also, given the recent role of fatty acids in stem cells differentiated into neural cells, we used of CLA to increase neural differentiation.

## Methods

### *Laboratory animals*

Male NMRI mice (25–30 g) and male Wistar rats (150–250 g), provided by Shahid Chamran University (Ahvaz, Iran), were housed in cages (22 ± 2°C under a standard 12-h light–dark cycle) and allowed *ad libitum* feed access. All experimental protocols were conducted according to the standards for animal care established by the institutional ethical committee.

### *Experimental design*

In this experimental study, 35 adult male Wistar rats were used. Behavioral experiments were carried out during the 12-h light period in a Y-shaped maze device. Animals were randomly divided into five groups, with seven rats per group: (i) a control group (trained in the Y-maze without surgery or treatment); (ii) a lesion group, which received a electrically induced lesion: 0.5 mA, 3 s (bilateral lesion of the nucleus basalis of Meynert [NBM] to create an Alzheimer's model); (iii) a sham group (the electrode was introduced to the NBM without current); (iv) a cell-therapy group (lesion + NLCs); and (v) a vehicle group (lesion + 0.1 mL phosphate-buffered saline [PBS]).

### *Stereotaxic surgery*

For stereotaxic surgery, rats were anesthetized by intraperitoneal injection of ketamine hydrochloride (78 mg/kg) and xylazine (3 mg/kg). To create an AD model, they were then placed in a stereotaxic apparatus. The animals' NBM was destroyed bilaterally with an electrical lesion (0.5 mA for 3 s). The electrode was aimed at the NBM with the incisor bar set at −3.3 mm below the interaural line according to the following coordinates from the stereotaxic atlas of anteroposterior: −1.30 mm from bregma, L: ± 2.8 mm both respect to bregma, and DV: −8 mm from cranium surface. One week after the bilateral lesion of the NBM, the neural precursor cell (0.1 mL) was injected via stereotaxic surgery using a Hamilton syringe at NBM coordinates, and behavioral tests were performed by the Y-maze task 5 weeks after the lesion.

### *Isolation and culture mouse embryonic fibroblast cells*

Mouse embryonic fibroblast cells (MEFs) were isolated and cultured by using an enzymatic method in 13-day-old embryos of NMRI mice. The isolated cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS), 2 mmol/mL l-glutamine and penicillin/streptomycin until passage 3. MEFs were inactivated via mitomycin C (Sigma) treatment and used as a feeder layer for mESCs.

### *Inactivation of embryonic fibroblasts by mitomycin C*

The confluent fibroblasts were incubated for 2 h with 2 mg/mL mitomycin C to inactivate their