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Standardizing Harvesting Methods for Human Turbinate Mesenchymal Stem Cells from Surgical Tissue

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Abstract

In accordance with many disease occurrences, stem cell research has become an important study. Mesenchymal stem cell (MSC) is one of representative materials of stem cell research. MSCs are multipotent progenitor cells that can differentiate into various cell types including osteocytes, adipocytes, chondrocytes, myocytes, stromal cells and neurons. MSCs have been derived from bone marrow, umbilical cord, umbilical cord blood, fat, and other body organs. Among them, human inferior turbinate and nasal septal cartilages are good materials for extraction of MSCs. For extraction of MSCs, we used MSC extraction protocol from ARCO sensorineural laboratory. In order to identified extracted MSCs, Immunofluorescence to CD90 and CD73 (or CD105) cell-surface markers of MSCs was performed using the MSC phenotyping kit (Miltenyi Biotec, Germany). hMSCs derived from inferior turbinate and nasal septal cartilage were shown positive results. Further study, more studies need to comparative analysis of differentiated target cells from our MSCs.

Keywords: Mesenchymal stem cell; HMSC; Human turbinate; Septal cartilage

Introduction

Mesenchymal stem cells are rare, multipotent progenitor cells that can differentiate into osteocytes, adipocytes, chondrocytes, myocytes, cardiomyocytes, fibroblasts, myofibroblasts, epithelial cells, and neurons. Mesenchymal stem cells have been derived from bone marrow, adipose tissue, umbilical cord, umbilical cord blood, and other body organs. But these procedures are highly invasive and not easy to master. The human inferior turbinate appears clearly in surgical view for ENT specialists and can be an excellent candidate donor site for mesenchymal stem cells. Many doctors have reported that mesenchymal stem cells from the turbinate have several advantages compared to other stem cells. For instance, mesenchymal stem cells have been successfully used for autologous transplantation for myocardial infarct, spinal cord trauma, hippocampal lesion defect and Parkinson's disease in mammalian animal models. These cells also provide excellent results in the repair of central nervous system and damaged nerve tissue.

Several studies report the therapeutic applications of nasal olfactory mucosa mesenchymal stem cells (OM-MSCs). But OM-MSCs have some limitations such as high susceptible to infection, scarcity, and damage during extraction procedures. Unlike OM-MSC, human turbinate mesenchymal stem cells (hTMSC) show more flexibility in extraction and cultivation, and resistance to infection, and can be extracted in large numbers. In this study, we investigated the presence of hTMSCs in inferior turbinate tissue collected from surgical procedures (inferior turbinectomy in chronic hypertrophic rhinitis patients). We adopted total turbinectomy as the surgical procedure of choice, since this procedure would improve the breathing and has a good surgical outcome. We characterized the natural course and multiplicity of these cells under the specific conditions employed by us. Finally, we compared the cells extracted following our procedure with those in published reports describing the extraction of turbinate mucosa partially. This study hence describes preliminary results for iPSC induction, neural differentiation, and hearing recovery using stem cells.

Materials and Methods

Collection of human tissues

Tissue samples were collected from the inferior turbinate of the nasal cavity. Additionally, septal cartilage was harvested by septoplasty. All the biopsies were performed under the agreement of "laboratory guideline and informed consent". Palatine tonsil and nasal polyp was collected during nasal surgery. The specimens collected are as follows: 29 inferior turbinate (bilateral) (Figure 1), 7 nasal septal cartilages (unilateral), 1 palatine tonsil (bilateral) and 3 polyps (unilateral) (Table 1).

Isolation of hTMSCs from tissues

Tissue samples from patients were washed 4 times using 2 × antibiotic-antimycotic (Biowest, France) in DPBS (Biowest, France). After washing, the tissue samples were cut into 3 mm × 3 mm pieces.

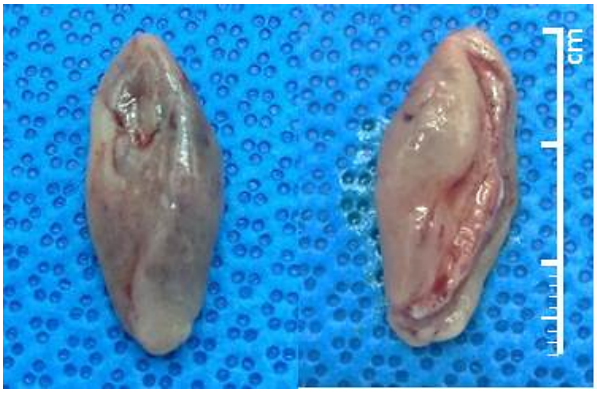

Figure 1: Human inferior turbinate acquired from total inferior turbinectomy surgery (length=2.8 cm).

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Patient No.	Sample No.	Age	Sex	Tissue	Patient No.	Sample No.	Age	Sex	Tissue
1	1-IT	40	M	Inferior turbinate	17	17-IT	57	M	Inferior turbinate
	1-P			Polyp	18	18-IT	51	M	Inferior turbinate
2	2-IT	18	M	Inferior turbinate	19	19-IT	45	M	Inferior turbinate
3	3-IT	31	F	Inferior turbinate	20	20-IT	29	M	Inferior turbinate
4	4-IT	45	F	Inferior turbinate	21	21-IT	48	F	Inferior turbinate
5	5-IT	33	M	Inferior turbinate	22	22-IT	30	M	Inferior turbinate
6	6-P	53	M	Polyp		22-C			cartilage
7	7-IT	46	M	Inferior turbinate	23	23-IT	54	F	Inferior turbinate
	7-P			Polyp	24	24-IT	27	F	Inferior turbinate
8	8-IT	56	M	Inferior turbinate	25	25-IT	21	M	Inferior turbinate
9	9-IT	31	M	Inferior turbinate	26	26-IT	20	M	Inferior turbinate
	9-T			Tonsil		26-C			cartilage
10	10-IT	51	F	Inferior turbinate	27	27-IT	27	M	Inferior turbinate
11	11-IT	27	M	Inferior turbinate	28	28-IT	23	M	Inferior turbinate
12	12-IT	43	M	Inferior turbinate		28-C			cartilage
13	13-IT	21	M	Inferior turbinate	29	29-IT	52	M	Inferior turbinate
14	14-IT	63	M	Inferior turbinate		29-C			cartilage
15	15-IT	43	M	Inferior turbinate	30	30-IT	49	M	Inferior turbinate
	15-C			cartilage		30-C			cartilage
16	16-IT	20	F	Inferior turbinate					
	16-C			cartilage					

Table 1: Details of samples collected for this study.

To isolate hTMSCs from the tissue (which consists of bone, soft tissue, mucosa, and blood), the samples were placed in a 35 mm collagen-coated culture dish and covered with a sterilized cover slide glass. We used the Dulbecco's modified Eagle's medium (DMEM, Biowest, France) containing 10% fetal bovine serum (FBS, Biowest, France) and 1 × antibiotic-antimycotic solution as culture medium. The small tissue samples were incubated at 37°C in an atmosphere of 5% CO_2. The culture media were changed once every 2 to 3 days. After 4 weeks, hTMSCs were detached using 1 ml of 0.05% trypsin-EDTA solution. The isolated hTMSCs were passage between 4 to 8 times for characterization and iPSC reprogramming.

Characterization of hTMSCs by immunofluorescence

Immunofluorescence analysis to detect CD90 and CD73 (or CD105) cell-surface markers was performed using the MSC phenotyping kit (Miltenyi Biotec, Germany). Briefly, after harvesting and counting the cells, the cell suspension was centrifuged at 300 × g for 10 min. The supernatant was removed completely and the cell pellet was resuspended in 100 µL of buffer (PBS containing 10% FBS and 2 mM EDTA). Next, 10 µL of MSC phenotyping cocktail (1:11) was added to the cells. The sample was mixed well and incubated for 10 min in the dark at 4°C. Stained cells were then washed with 1 ml of buffer and centrifuged at 300 × g for 10 min. The supernatant was discarded and the cell pellet was resuspended in 100 µL of buffer. Fluorescence corresponding to CD90 and CD73 (or CD105) was observed microscopically (EVOS XL, Thermo Fisher).

Results

Isolation of hMSCs

For isolation of hMSCs, surgically obtained tissue samples were incubated immediately after inferior turbinectomy. Since our laboratory is located on the 3rd floor of the hospital, while the operation room is located on the 4th floor, most of the samples were incubated for less than 30 min. As hMSCs were derived from various tissues such as inferior turbinate, nasal cartilage, tonsil and nasal polyp, the first stage of differentiation was observable after 7 days of incubation (Figure 2). At initial time points of culturing, cells around tissues such as turbinate, tonsil, and nasal polyps were shaped differently from MSCs, whereas spread cells were similar into MSCs. Notably, cells from nasal cartilage resembled MSCs from the beginning. After 4 weeks in culture, cells derived from the sample tissues were harvested from the culture dishes, using 0.05% trypsin-EDTA solution, and passaged. The passaged hMSCs formed colonies as shown in Figure 3.

Characterization of isolated hMSCs

Immunofluorescent staining of hMSCs for cell-surface markers such as CD73, CD90 and CD105 was performed for characterization. The result of immunostaining revealed that cells derived from the interior turbinate were positive for CD73 and CD90 (Figure 4), which is a characteristic phenotype of MSCs. Positive results were also seen for cells derived from other tissues such as nasal cartilage, tonsil, and nasal polyp. These results suggested that cells derived from inferior turbinate, nasal septal cartilage, palatine tonsil, and nasal polyp were indeed hMSCs.

Comparison with conventional methods of extracting hMSCs

We found that there were significant differences between the method

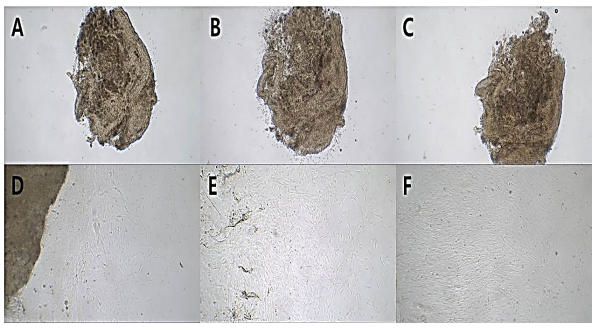

Figure 2: Cell growth at different time points. A part of human turbinate for isolation of hMSCs (A). Cells were not observed after 3 days (B) and 5 days (C) of incubation. After 7 days, cultured cells were observed (D). The obtained hMSCs were expanded in cell culture dish after 2 weeks (E) and 4 weeks (F).

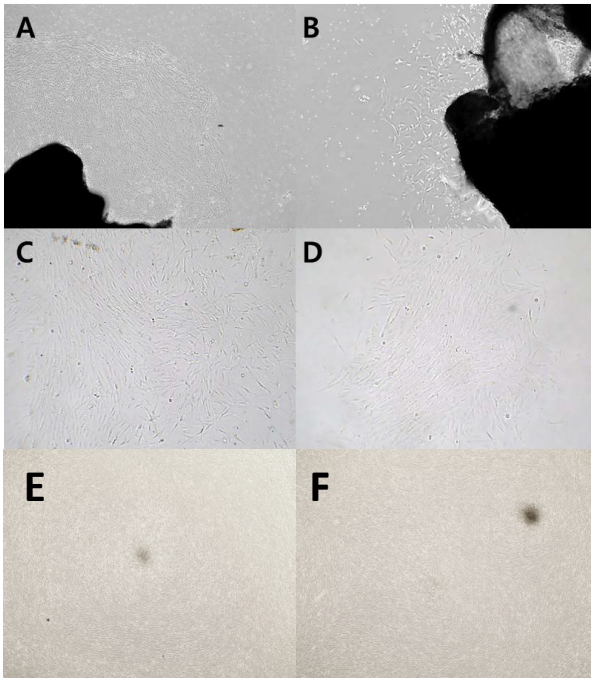

Figure 3: Morphology of cells derived from tissues. A. Cells derived from inferior turbinate after 7 days. These cells were differently shaped than MSCs. B. Cells derived from nasal cartilage of same donor after 7 days. C. After 20 days of culturing, cells resembled MSCs. D. Colony of hMSCs in culture.

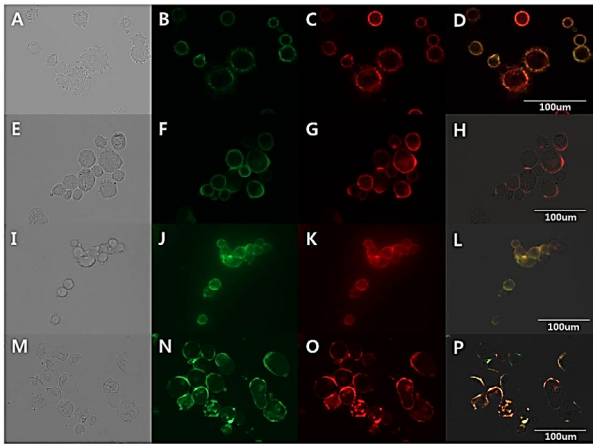

Figure 4: Immunofluorescent staining of hMSCs derived from inferior turbinate (A-D), palatine tonsil (E-H), nasal polyp (I-L), and nasal septal cartilage (M-P). Panels from left to right represent the trans, CD90 (GFP), CD73 or CD105 (RFP) and Merge (Scale=00 μm).

applied by us to extract MSCs in this study and other published studies that deal with the extraction of MSCs from human and animal sources. Most published procedures extract only a small amount of tissue from the mother organ, whereas we extracted a considerable amount of tissue from donor site in this study. Further, although we were unable to find the exact time interval between extraction procedures and incubation in published studies, this time interval is likely to be between 3 and 24 h, in contrast to not more than 30 min in our study.

Discussion

Although cells from skin tissue are considered good candidates for iPSC induction, hTMSCs have several advantages over human dermal fibroblast, such as easy accessibility and fewer complications during surgery (lesser bleeding, donor site defect and easy availability of tissue during nasal surgery) [1]. In addition, hTMSCs have some unique characteristics such as easy accessibility, high differentiation rates and good survival rate [2]. In this study, we have tried to optimize the extraction of hTMSCs for the study of iPSC induction and neuronal differentiation from tissues obtained from the nasal cavity during surgical procedures.

Many researchers have reported the presence of olfactory mucosa in hTMSCs, originating from the olfactory nerve, i.e. CN no. 1 (cranial nerve number 1), which is the only exposed cranial nerve. Being exposed, the olfactory nerve is a highly vulnerable organ and damage to this nerve results in hyposmia and anosmia in a large number of patients. Although hTMSCs are promising candidates for iPSC induction or as donor cells for such patients, only a few studies using hTMSCs have been reported [1,3]. An important road block currently faced is the low success rate of stem cell differentiation and iPSC induction. Our study may help address this issue as by using hTMSCs for reprogramming, it is possible to get the unique characteristics of neuronal cells and solve the problem of nerve regeneration. This may be due to the fact that olfactory cells secrete unique materials that promote neural differentiation [4].

Since the discovery of iPSCs by Takahashi et al. [5], many researchers have explored the possibilities of self-renewal and regeneration of iPSC, embryonic stem cells, and adult stem cells, using TMSC technology [6-8]. Indeed, Takahashi et al. not only introduced the iPSC technique but also neural differentiation in their study [5]. The various methods being currently employed in this area each have their own pros and cons and are the targets of active study by stem cell researchers. It is our opinion that regardless of these handicaps, it is crucial to find an optimal recipient of stem cells. Cell reprogramming techniques may indeed provide treatment for diseases that are difficult to manage, such as allergic disorders [9]. Finally, we suggest that our technique for hTMSC extraction and culturing and the data presented in this study will provide an efficient method for the extraction of stem cells.

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