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Mesenchymal Stem Cells (Mscs) in Scanning Electron Microscopy (SEM) World

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Original Investigation

Abstract

Objectives: Adipose and bone marrow-derived mouse mesenchymal stem cells (MSCs) were isolated, and the method to bring these cells to scanning electron microscopy (SEM) was described in this study. This paper describes the protocol for SEM and defines the outer cell shape and interactions between the cells.

Methods: In this study, adipose and bone marrow-derived mouse MSCs were isolated on coverslips through a method with a procedure specified for SEM.

Results: Basically, by SEM, only the pseudopods arising from each cell and extending through each other were clearly shown in this study. Cell-to-cell interactions (signalization) play an important role in the reproduction of cells within the flask, and it is believed that the cells communicate with each other; thus, monolayer confluence occurs.

Conclusion: In this study with SEM, the outer surface of cells was investigated, and the pseudopods were also shown to extend through each other.

Key words: Scanning electron microscopy (SEM), mesenchymal stem cells (MSCs), mice, veterinary medicine

Introduction

Stem cells have the unique potential to generate many differentiated tissue cells under the appropriate conditions in an organism (1). Stem cells are being widely used in many diseases. Among them are diabetes, cartilage disorders, osteoporosis, tendon and ligament injuries, neurodegenerative disorders, and cardiac failure. MSCs can be found in numerous tissues; these fibroblast-like, multipotent adult stem cells are capable of differentiating into different lineages (osteogenic, chondrogenic, adipogenic lineages). Stem cell therapies in veterinary medicine focus on tendon and ligament injuries, cartilage and joint diseases, muscular dystrophy, wounds, burns, and atopic dermatitis (2, 3, 4). Mesenchymal stem cells are fibroblast-like cells, and these cells could be differentiated into skeletal muscle, endothelium, and neuronal cells (5).

Due to the ease of obtaining them and the immunosuppressive effects of MSCs, they are widely used in regenerative medicine (3). Fridenstein, for the first time in 1976 by using fetal calf serum, showed that cultures of bone marrow MSCs are capable of adhesion in flasks, and these fibroblast-like colonies could differentiate into bone and adipose tissue (6). He also showed that the limited number of these cells within tissues could be replicated by using a culture medium.

It has been shown in transmission electron microscopy (TEM) studies that MSCs contain a large euchromatic nucleus, prominent nucleolus, GER and numerous free ribosomes, numerous mitochondria with cristae, and organelle-rich active cell structure. In adipogenic differentiation, large well-developed lipid droplets and numerous mitochondrial granulated ER are observed, while in chondrogenic and osteogenic differentiation, GER and abundant free ribosomes are observed (1, 7). In TEM examination of MSCs, pseudopod-like structures have also been observed (5). The main role of these structures is to transmit the produced material from one cell into another by extending the pseudopods to a point that the vacuoles are produced (5). In an SEM study of rat adipocyte cells, the culture was examined, and the cell surface properties, cell shape, cytoskeletal matrix (CSM), and extracellular matrix (ECM) properties were reported (8).

In this study, we aimed to investigate MSC interactions with each other through their extensions by SEM.

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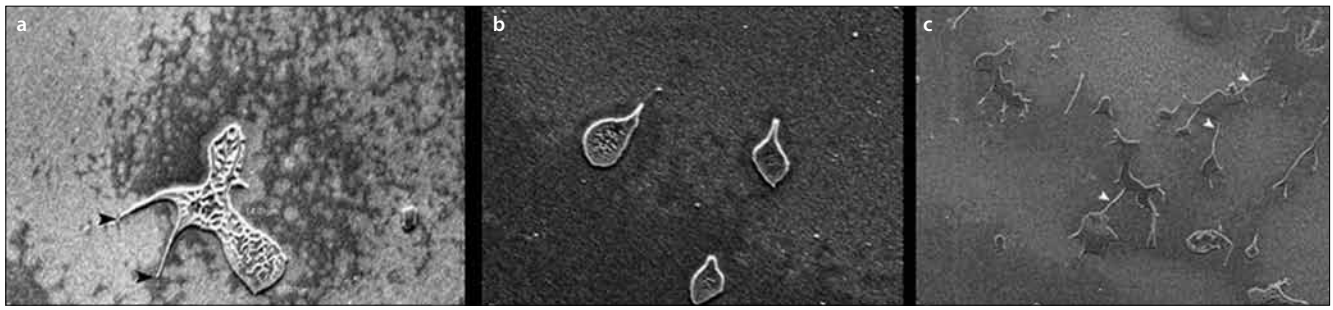

Figure 1. SEM photos of mouse mesenchymal stem cells derived from adipose tissue (a, b, c)

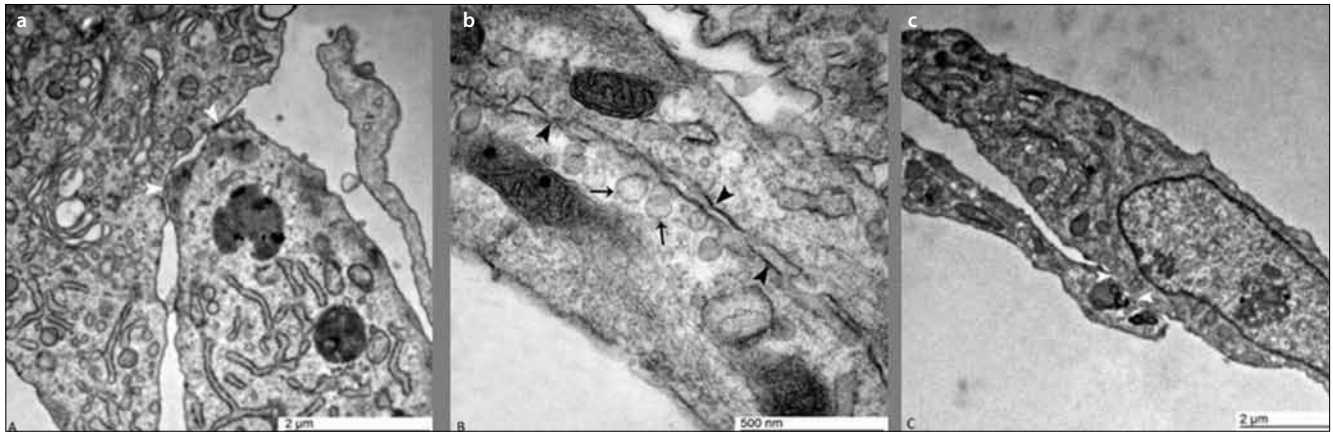

Figure 2. TEM photos of horse (a) and sheep (b, c) mesenchymal stem cells derived from bone marrow

Methods

Adipose and bone marrow-derived mouse MSCs were isolated on coverslips through a method described below, with the procedure specified for SEM (9).

Cells attaining 70% confluence on 0.2% gelatin-precoated 10-mm coverslips in 35-mm petri dishes were induced for differentiation. Dishes without treatment were used as controls and were kept for the same period of induction using normal basic maintenance medium. After 14 days of treatment, the dishes containing the coverslips with differentiated cells were used for SEM analysis. In preliminary experiments, differentiated adipocytes were detached, and lipid was extracted by normal fixation and dehydration procedures used for SEM (10). Unique procedures were developed for handling, fixing, and dehydrating. Treatment medium from the dishes was decanted, and the cells were fixed with freshly prepared 2.5% glutaraldehyde (Sigma-Aldrich-G5882) in Dulbecco's phosphate-buffered saline (DPBS) (GIBCO, Life Technologies, Carlsbad, CA, USA) at 4°C for 3 hours. The dishes with fixed cells were washed twice with DPBS and dehydrated with methanol. Dehydration was carried out sequentially in the dishes with methanol at concentrations of 20%, 40%, and 60% for 5 min each, followed by an 80% methanol wash for 3 min and then a 100% methanol wash for 30 s, repeated five times. Coverslips with dishes were then dried in vacuum-assisted desiccators overnight and then stored at room temperature until SEM analysis was carried out. The surface of the coverslip was sputter-coated in a vacuum with an electrically conductive 5-nm-thick layer of gold-palladium alloy with the Precession Etching Coating system. SEM images were then recorded with a scanning electron microscope (Jeol 7000F) at a lower voltage (1 kV) and low vacuum mode with a tilt of 30.

Results

Scanning electron microscopy (SEM) micrographs provide information about the mineral structures deposited on the surface of MSCs, cell surface connections, and formation of cell surface structures. In this study, extensions of the cells through each other could be clearly visualized (Figure 1a, b, c arrows). In our previous TEM study, we observed that in the extension points of the cells, abundant vacuoles are visible (Figure 2a, b, c arrows) (5). By SEM, only the outer surface of the mesenchymal cells could be observed; thus, in this research, the pseudopods arising from each cell and extending through each other were clearly shown.

Discussion

In a study that was conducted by light and confocal microscopy and TEM, MSCs were examined for morphological features (11). In confocal microscopic analysis of differentiated MSCs into adipocytes, abundant intracellular lipid droplets were observed. MSC cytoplasm contains two different stains: a basophilic and eccentric localized nucleus was found in the intensely stained section of the cell, whereas in the peripheral zones, the stain was pale. The cell membrane was irregular, and it is noted that the cells were lacking of pseudopods. In TEM, it was observed that small pseudopods were surrounding the cells.

It is believed and reported that small pseudopods visualized in MSCs allow the migration of cells within the tissue (11). In anoth-

er study conducted by TEM, bone marrow-derived MSC differentiation into adipocytes, osteocytes, and chondrocytes and microstructural changes due to differentiation were examined (12).

Pasquinelli et al. (13) have compared the fine structure of human bone marrow-derived MSCs with amnion- and chorion-derived MSCs. In human bone marrow-derived MSCs, expansion in the GER vesicles was observed, and the vesicles were localized in the periphery.

Vesicles at the periphery of the collection are believed to be related with the niche microenvironment.

Compared to bone marrow-derived MSCs, the fine structure of chorion-derived MSCs is found to be more basic and metabolically less active. GER and glycogen are reported to be accumulated in the periphery. In our study with TEM, we have reported that the vacuoles have accumulated in the cell-contact areas, and the peripheral region of membranes contains numerous vacuoles; these findings support the cell signalization (5).

Conclusion

In this study with SEM, the outer surface of the cells was investigated, and the pseudopods were also shown to extend through each other. We believe that these pseudopods could allow the migration of MSCs within the tissue.

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