Properties of Dental Pulp–derived Mesenchymal Stem Cells and the Effects of Culture Conditions

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Abstract

Dental pulp mesenchymal stem cells (DPMSCs) highly express mesenchymal stem cell markers and possess the potential to differentiate into neural cells, osteoblasts, adipocytes, and chondrocytes. Thus, DPMSCs are considered suitable for tissue regeneration. The colony isolation method has commonly been used to collect relatively large amounts of heterogeneous DPMSCs. Homogenous DPMSCs can be isolated by fluorescence-activated cell sorting using antibodies against mesenchymal stem cell markers, although this method yields a limited number of cells. Both quality and quantity of DPMSCs are critical to regenerative therapy, and cell culture methods need to be improved. We thus investigated the properties of DPMSCs cultured with different methods. DPMSCs in a three-dimensional spheroid culture system, which is similar to the hanging drop culture for differentiation of embryonic stem cells, showed upregulation of odonto-/osteoblastic markers and mineralized nodule formation. This suggests that this three-dimensional spheroid culturing system for DPMSCs may be suitable for inducing hard tissues. We further examined the effect of cell culture density on the properties of DPMSCs because the properties of stem cells can be altered depending on the cell density. DPMSCs cultured under the confluent cell density condition showed slight downregulation of some mesenchymal stem cell markers compared with those under the sparse condition. The ability of DPMSCs to differentiate into hard tissue–forming cells was found to be enhanced in the confluent condition, suggesting that the confluent culture condition may not be suitable for maintaining the stemness of DPMSCs. When DPMSCs are to be used for hard tissue regeneration, dense followed by sparse cell culture conditions may be a better alternative strategy. *(J Endod 2017;■:1–4)*

Key Words

Cell density, colony isolation method, culture condition, dental pulp mesenchymal stem cells, endodontics, pulp regeneration, 3D spheroid culture

Significance

Cell culture conditions of DPSCs modify their properties. Dense cell culture conditions downregulate some mesenchymal stem cell markers and proliferation activity and promote hard tissue–forming cell differentiation.

Stem cells used for regenerative medicine are composed of pluripotent stem cells, including embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, and multipotent stem cells, such as adult or somatic stem cells. ES and iPS cells possess the pluripotent capacity to differentiate into various types of cells; therefore, they are inherently associated with the risk of tumorigenesis (1). On the other hand, somatic stem cells, including mesenchymal stem cells (MSCs), are not pluripotent and can differentiate into only several types of cells; the risk of tumorigenesis from the use of somatic stem cells is anticipated to be lower (2). Regenerative therapy is generally performed by using MSCs (3, 4), which are derived from various mesenchymal tissues and organs such as the bone marrow, adipose tissue, synovium, and muscles (5). Dental pulp cells are a combination of migrating cranial neural crest cells and mesenchymal cells within the first branchial arch (6), and stromal stem cells isolated from dental pulp tissues belong to MSCs. Dental pulp mesenchymal stem cells (DPMSCs) were first isolated from the pulp tissues of human third molars and were designated as postnatal dental pulp stem cells (DPSCs) (7). DPMSCs selected on the basis of high growth rate and high expression of MSC markers, such as CD44, 90, 105, and 146, demonstrated the potential to differentiate into neural cells, osteoblasts, adipocytes, and chondrocytes (5). Thus, DPMSCs are considered suitable for regenerative therapy, although they basically possess the ability to form a dentin/pulp-like complex (7).

Isolation Methods of DPMSCs

Human DPMSCs are commonly obtained from wisdom teeth or primary teeth, which are usually discarded (5). They can also be obtained from supernumerary teeth. The amount of stromal stem cells distributed in organs and tissues is small; percentages of MSCs are reported to be 0.01%–0.001% of the total nucleated cells in bone marrow (8). The number of DPMSCs in dental pulp tissues is also small. Several methods have been reported to isolate DPMSCs from pulp tissues. Bone marrow stem cells (BMSCs) are usually isolated by using the colony isolation method; BMSCs are obtained from the colonies formed from the attached bone marrow stromal cells on culture dishes (8, 9).

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DPSCs are isolated by using this method (7). The advantages of this method include its easy implementation and high performance (high cell yield). In particular, the latter is important for clinical application because a large number of stem cells are usually required for regenerative therapy. Indeed, a sufficient number of synovial MSCs are isolated by using the colony isolation method to be used for therapeutic application (10). However, the isolated stem cells using this method are heterogeneous because the size of each colony is not the same, suggesting that each cell clone may have a different growth rate, and different cell morphologies and sizes may be observed within the same colony (11). In other words, the "true" stem cells included in the stem cells isolated by using the colony isolation method may represent the stem cell properties.

We evaluated the properties of DPMSCs isolated from dental pulp tissues of human third molars by using the colony isolation method (n = 5; age, 18–34 years; approved by the Ethics Committee of the Faculty of Dentistry, Tokyo Medical and Dental University [#948]). Most of the DPMSCs expressed typical MSC markers such as CD44, CD73, CD90, and CD105 but were negative for CD34 (hematopoietic progenitor cell antigen). Of the total DPMSCs, 60%–70% were CD146+ DPMSCs. These results indicate that the colony isolation method can be used to isolate DPMSCs with different mesenchymal markers but cannot be used to isolate a homogenous population of DPMSCs.

Homogeneous stem cell populations of DPMSCs can be isolated by using fluorescence-activated cell sorting (FACS) or magnetic beads and specific antibodies such as CD105 and STRO-1 (12, 13). Purified CD105+ DPMSCs show a higher differentiation capacity compared with DPMSCs isolated by using the colony isolation method (14). However, the FACS or magnetic beads isolation methods showed limited yields of DPMSCs. Therefore, a long culture time is required to obtain a sufficient number of DPMSCs.

Recently, a membrane isolation method using a specific chemotactic factor has been reported (15). Granulocyte colony-stimulating factor, a chemoattractant for DPMSCs, was used to isolate homogenous DPMSCs from dental pulp cell suspensions (15). This method is simple and easy to apply, and collected stem cells using this method possess high angiogenic/neurogenic and regenerative potentials, which are independent of age (16, 17). However, a disadvantage of this method is the limited cell yield.

Three-dimensional Spheroid Culture of DPMSCs

DPMSCs isolated by using the colony isolation method or other methods possess the capacity to differentiate into several different types of cells composed of dental pulp, bone, cartilage, nerve, muscle, and blood vessels (5, 18, 19). What are the natural properties of DPMSCs when they are located in the stem niche of the pulp tissue? An *in vitro* two-dimensional (2D) culture system allows the expansion of cells on culture dishes. However, cells constructing tissues or organs are present in three-dimensional (3D) conditions such as in the human body. Therefore, 3D culture may be useful to reveal the original properties of DPMSCs *in vivo*. We cultured DPMSCs isolated by using the colony isolation method in low-attachment culture plates to induce spheroid bodies. DPMSCs cultured in 3D spheroid bodies show higher expression of odonto-/osteoblastic markers, including alkaline phosphate, osteocalcin, and dentin sialophosphoprotein, than 2D monolayer cultured DPMSCs. Mineralized nodule formation was also induced by culturing in odonto-/osteogenic differentiation medium containing L-ascorbic acid phosphate magnesium salt, ß-glycerophosphate, and dexamethasone. Mineralized nodules rapidly formed in 3D spheroid cultured DPMSCs compared with 2D monolayer cultured DPMSCs (Fig. 1). These results suggest that DPMSCs may possess a hard tissue–forming capacity *in vivo*, although they were allowed to differentiate in the odonto-/osteogenic differentiation mediums or culture conditions. Enhanced cell-cell and cell–extracellular matrix (ECM) interactions among 3D spheroid cultured cells may be a potent inducer of odonto-/osteoblastic differentiation, promote integrin signaling, a major adhesion molecule, and may be involved in the induction of odonto-/osteoblastic marker expression (20).

Effect of Cell Culture Density on the Properties of DPMSCs

The expansion of DPMSCs by culturing and passaging is necessary before their transplantation, because the amount of DPMSCs obtained from dental pulp tissue may not be sufficient for tissue regeneration. During cell expansion, the properties of stem cells can be easily affected

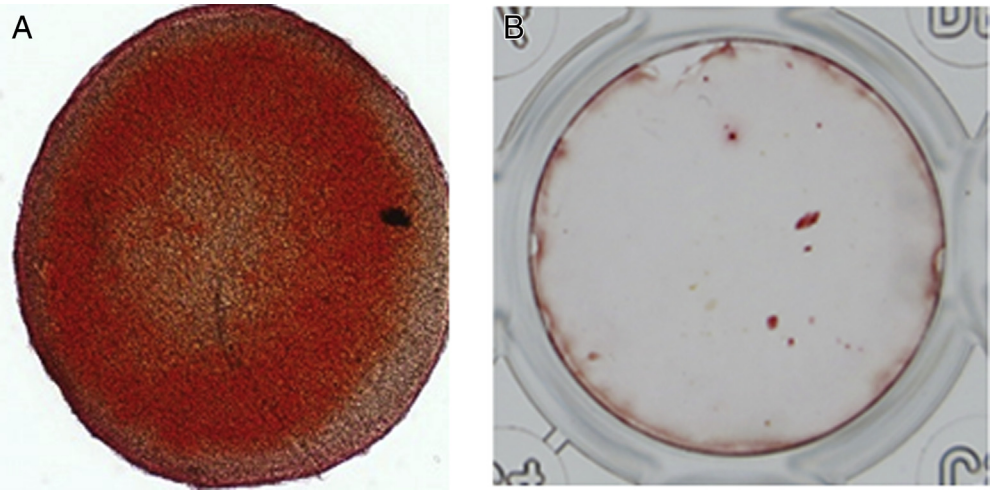

Figure 1. Mineralized nodule formation of 2D monolayers and 3D spheroid cultured human DPSCs. Human DPSCs were cultured in mineralization medium containing L-ascorbic acid phosphate magnesium salt, β-glycerophosphate, and dexamethasone, and mineralized nodules were detected by alizarin red S staining. Mineralized nodules were rapidly formed in 3D spheroid cultured DPSCs at 3 days (*A*). Diameter of spheroid was around 0.8 mm. On the other hand, mineralized nodules were rarely observed in 2D cultured DPSCs at 7 days (*B*).