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# Discovery of a novel type of Multipotent Dental Papilla Stem Cells (MDPSCs) in Three−Dimensional (3D) Spheroid Culture using RNA sequencing

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The ease of isolation of dental pulp stem cells (DPSCs) from discarded or extracted teeth offers a perfect source as a type of autologous stem cells. However, consensus is still lacking pertaining to identification of DPSCs and it is suggested that subpopulations of DPSCs coexist in dental pulp. Hence, efforts are ongoing in exploring unique surface markers of DPSCs. As is known, two−dimensional (2D) culture cannot recapitulate the environment of stem cells in the body, as a result, surface markers of stem cells will change and the cells tend to lose their stemness gradually. Compared to 2D culture, 3D culture of cells ensures original traits of stem cells to be preserved [1].

In past years, various 3D culture methods have been introduced to overcome the limitations of *in vitro* 2D culture and better mimic *in vivo* conditions. Recently, a new 3D culture method called spheroid culture has been developed. 3D spheroid culture facilitates enrichment of stem cells with high level of stemness. It is therefore considered to be a powerful tool to research into the behavior of stem cells. Empowered by this culture method, Chen *et al*. discovered a novel sub−group of multipotent stem cells from dental papilla [2]. They isolated and digested dental papilla from molar tooth germ of neonatal mouse and cultured them in stem cells growth media monolayer culture. Dental papilla cells (DPC, passage number two : P2) were inoculated into ultra−low attachment plates to facilitate formation of DPC spheres [Figure 1]. RNA sequencing of cells retrieved from three culture methods (monolayer culture, DPC sphere, DPC P2) was conducted to compare differentially expressed genes among three groups. GO analysis indicated that calcification related genes were significantly enhanced in DPC spheres. Signaling pathway analysis revealed Wnt and PI3K were the top two pathways that were activated in DPC spheres culture. Flow cytometric analysis elucidated top fifteen candidate surface markers that were expressed in DPC spheres as compared to cells from monolayers cultures. Further, RT−qPCR and western blot were used to verify the expression of surface markers and CD24a was confirmed to be highly expressed both in mRNA and protein form than monolayer culture. Importantly, CD24a was positively related to sphere forming efficiency of cells. Next, Chen *et al*. explored the expression levels using RNA sequencing and discovered Sp7 (a transcription factor of osteoblast differentiation) was highly expressed in DPC spheres. They used shRNA to knock down the expression of *Sp7* and found the sphere formation ability was significantly reduced due to silencing of *Sp7* gene [Figure 2]. Based on those results, they concluded that a new type of multipotent stem cells ($CD24a^+$) was discovered and that proliferation of this $CD24a^+$ positive multipotent cells was dependent on Sp7 expression. Finally, cells from DPC spheres, DPC P2 and monolayer cultures were transplanted into immunocompromised mice for four weeks. It was observed that only in the DPC spheres group

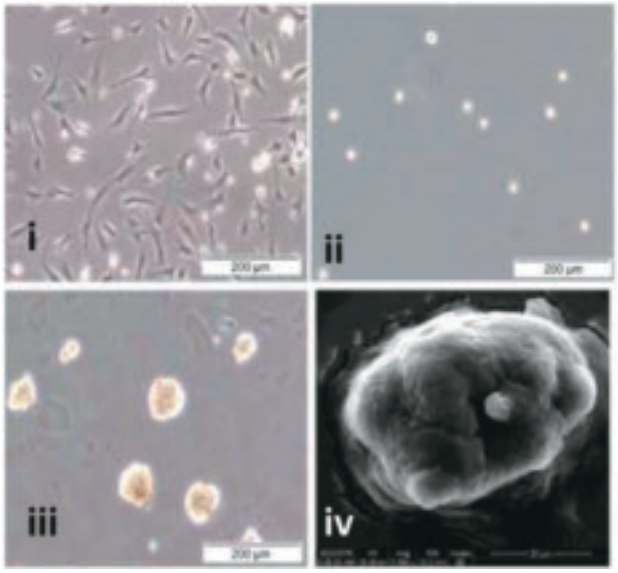


**Figure 1.** Cell morphology. i : DPC P2 ; ii : DPC spheres day 0 ; iii : DPC spheres day 7 ; iv : SEM photo of DPC spheres. Scale bar : 200 μm. The fours photos were extracted from reference 2.

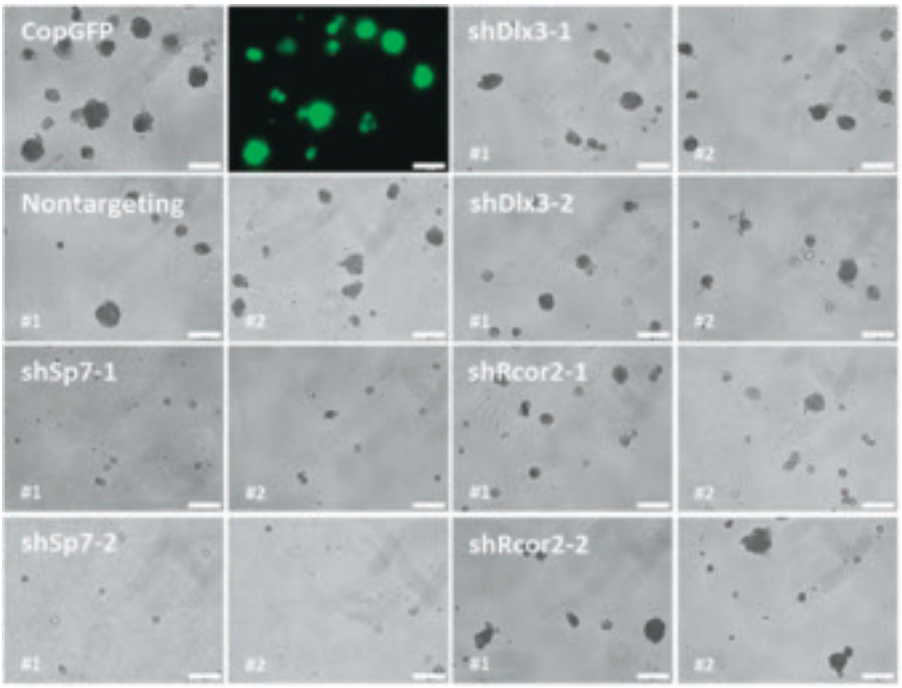


**Figure 2.** Knockdown of Sp7 leads to significant decrease of sphere formation from primary DPCs.

did the pulpo–dentinal complex–like structures regenerated.

Taken together, this study uncovered a new type of multipotent stem cells existent in dental papilla and shed novel insight into our understanding of the dentin regeneration. This work indicates that spheroid culture is a promising culture method of stem cells and RNA sequencing technology could be a powerful tool in exploring novel stem cell surface markers.

**Reference :**

[1] Cesarz Z, Tamama K. Spheroid culture of mesenchymal stem cells. Stem Cells Int. 2015 Apr 3. Article ID : 9176357. doi : org/10.1155/2016/9176357.

[2] Chen H, Fu HC, Wu X, Duan YF, Zhang SC, Hu H, Liao YS, Wang T, Yang Y, Chen GQ, Li ZH, Tian WD. Regeneration of pulpo–dentinal–like complex by a group of unique multipotent CD24a$^{+}$ stem cells. Sci Adv. 2020 Apr 8 ; 6 : eaay1514. doi : 10.1126/sciadv.aay1514.