CHAPTER 4

RESULTS

1. Morphology of cultured human DPCs and APCs after 3Mix treatment

After cell extraction with enzymatic technique, the first day revealed cells aggregated and attached on the culture plate surface. Cells gradually moved from minced tissue, aggregated and attached three to five days after cell extraction. Spindle-shaped or fibroblast-like cell were dominant in all population. On the 7th day, cells continued to proliferate and grow. Most of DPCs and APCs were fibroblast-like cells (Figure 27).

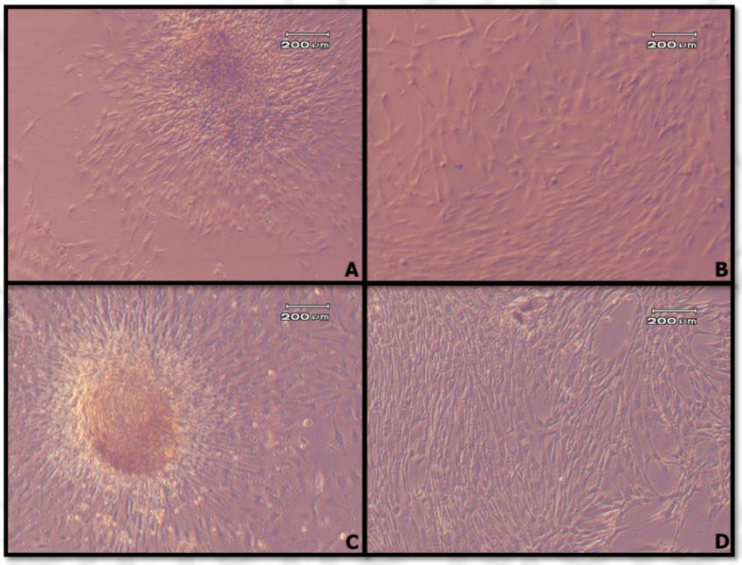

Figure 27: *Monitoring of cultured human DPCs and APCs under an inverted-light microscope. DPCs after three days of enzymatic extraction and cells gradually accumulated and attached (A). On the 7th day after extraction, DPCs continued to proliferate and grow with predominant fibroblast-like cells (B). APCs on days 3 (C) and days 7 after extraction (D) (40X magnification).*

The observation of cultured human DPCs morphology under an inverted-light microscope revealed DPCs showed polymorphic in shape mostly polygonal appearance. On the contrary, APCs culture exhibited smaller in size compared to DPCs, spindle or stellate in shape with numerous cytoplasmic process (Figure 28).

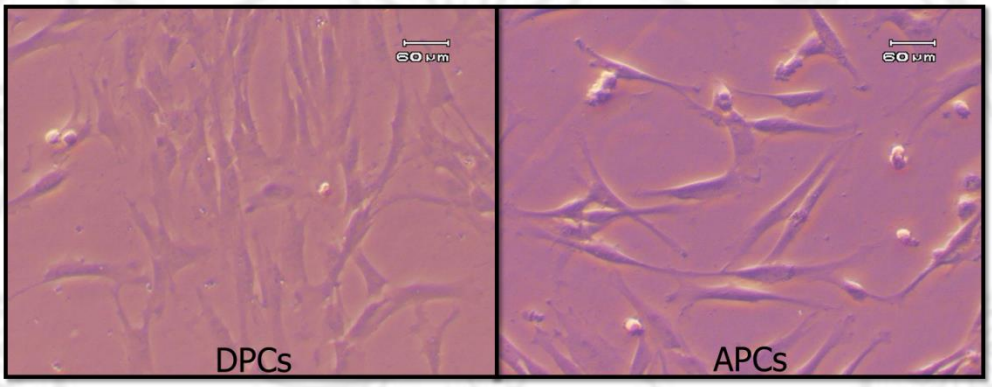

Figure 28: *Cultured human DPCs and APCs morphology under an inverted-light microscope. DPCs (A) showed polymorphic in shape mostly polygonal appearance. On the contrary, APCs culture exhibited smaller in size compared to DPCs (100X magnification).*

1.1 Morphology of cultured DPCs compared to 3Mix-treated DPCs

After DPCs were treated with 0.39 µg/ml 3Mix for 7 days, cell morphology was observed using H&E disclosing method under inverted-light microscope on days 7, 14, and 21 after treatment with 3Mix (Figure 29). Both cells in control groups and experimental groups reached their confluence on the 7th day after 3Mix removal and started to form a multi-layer afterward. There were no significant changes in their cell morphology and cell number in 3Mix-treated groups compared to control groups. Besides, Spindle-shaped cells were dominant in both groups.

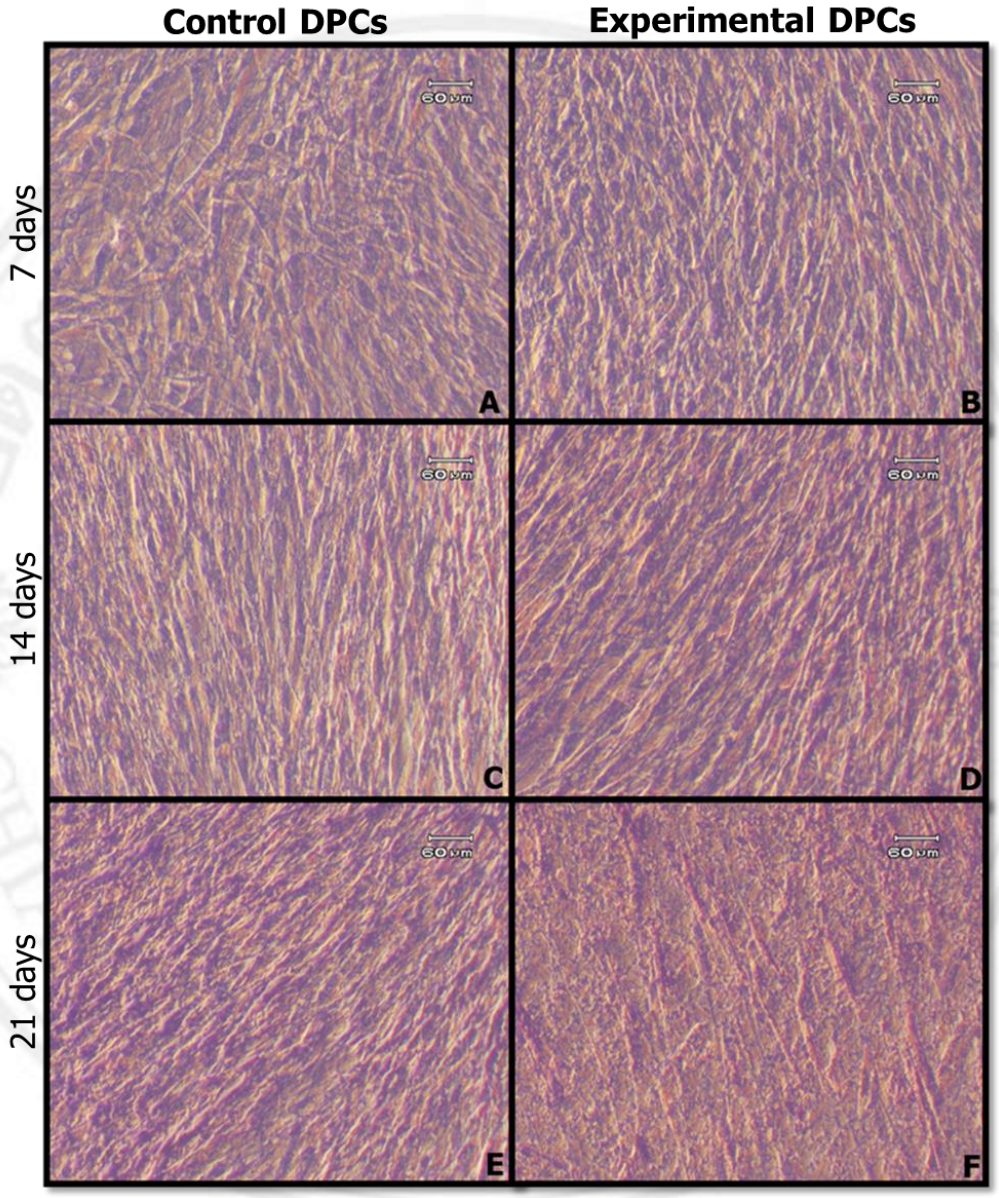

Figure 29: *Monitoring of cultured human DPCs under an inverted-light microscope. Control group of DPCs cultured in differentiating media for 7days (A). Experimental group of DPCs cultured in differentiating media for 7 days (B). Control group of DPCs cultured in differentiating media for 14 days (C). Experimental group of DPCs cultured in differentiating media for 14 days (D). Control group of DPCs cultured in differentiating media for 21 days (E). Experimental group of DPCs cultured in differentiating media for 21 days (F) (40X magnification).*

1.2 Morphology of cultured APCs compared to 3Mix-treated APCs

After APCs were treated with 0.39 µg/ml 3Mix for 7 days, cell morphology was observed using H&E disclosing method under inverted-light microscope on days 7, 14, and 21 after treatment (Figure 30). Both cells in control groups and experimental groups reached their confluence on the 6^{th} day after 3Mix removal and started to form a multi-layer afterward. There were no significant changes in their cell morphology and cell number in 3Mix-treated groups compared to control groups. Besides, Spindle-shaped cells were dominant in both groups.

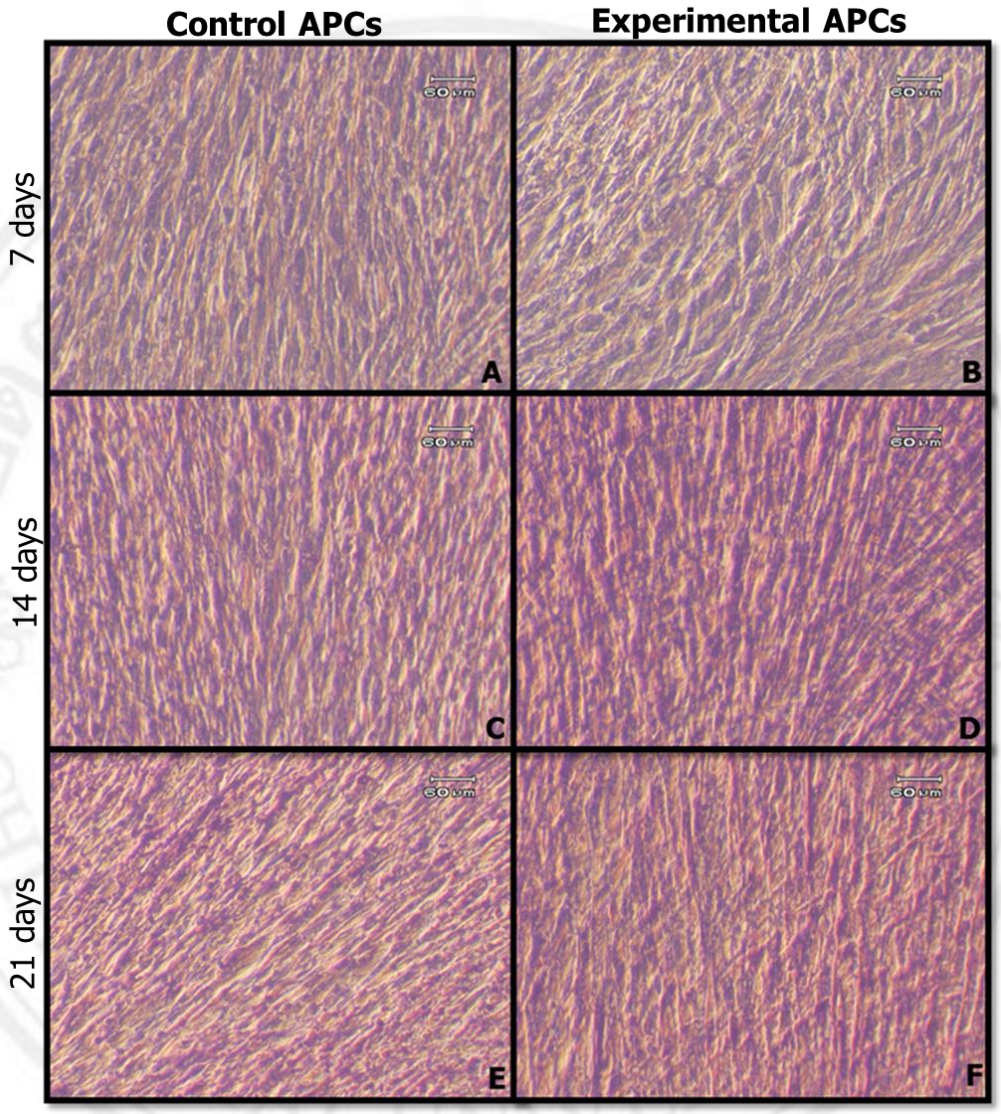

Figure 30: *Monitoring of cultured human APCs under an inverted-light microscope. Control group of APCs cultured in differentiating media for 7days (A). Experimental group of APCs cultured in differentiating media for 7 days (B). Control group of APCs cultured in differentiating media for 14 days (C). Experimental group of APCs cultured in differentiating media for 14 days (D). Control group of APCs cultured in differentiating media for 21 days (E). Experimental group of APCs cultured in differentiating media for 21 days (F) (40X magnification).*

2. Proliferative capacity of 3Mix-treated and untreated DPCs/APCs

There were significant differences (P<0.05) in proliferative capacity of DPCs at all time points in experimental group (0.39 µg/ml 3Mix treatment for seven days prior to assessment) compared to the control group. To clarify the ratio of cell proliferation capacity, the percentages of 3Mix-treated DPCs/untreated DPCs were 81.94%, 80.17%, 78.21% and 86.48% at days 1, 3, 5, 7 after treatment, respectively (Figure 31).

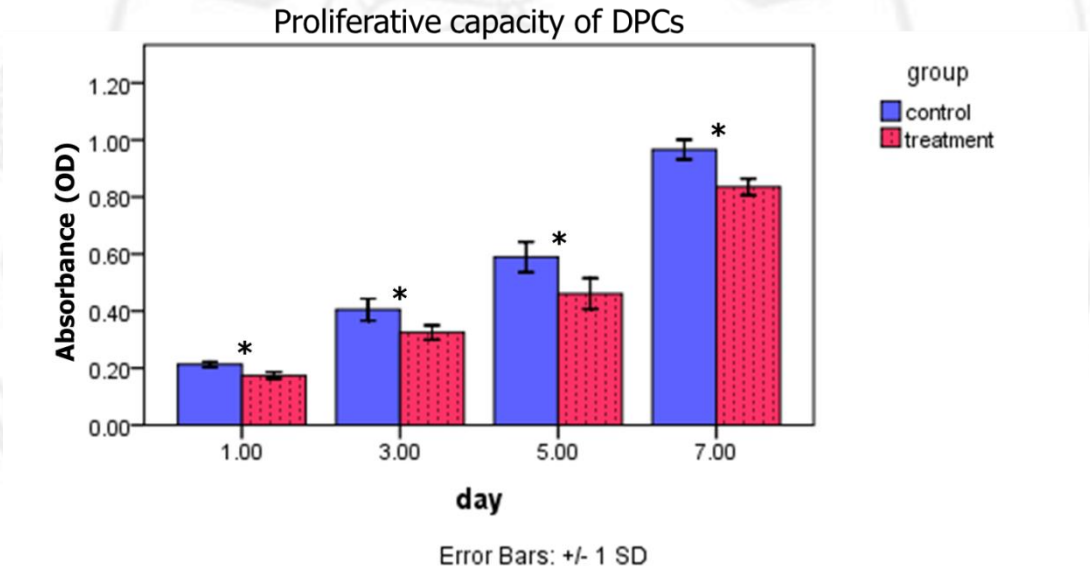

Figure 31: *The proliferative capacity of treated DPCs and untreated DPCs. There were statistically significant differences (*; P<0.05) in proliferative capacity of DPCs at all time points in the 3Mix-treated group compared to control group (Data were presented in Mean+/-SD).*

The proliferative capacity of treated APCs in experimental group (0.39 µg/ml 3Mix) also had significant differences (P<0.05) in proliferative capacity at all time points compared to the control group. The percentages of 3Mix-treated

APCs/untreated APCs were 81.26%, 80.69%, 83.39% and 90.37% at days 1, 3, 5, 7 after treatment, respectively (Figure 32).

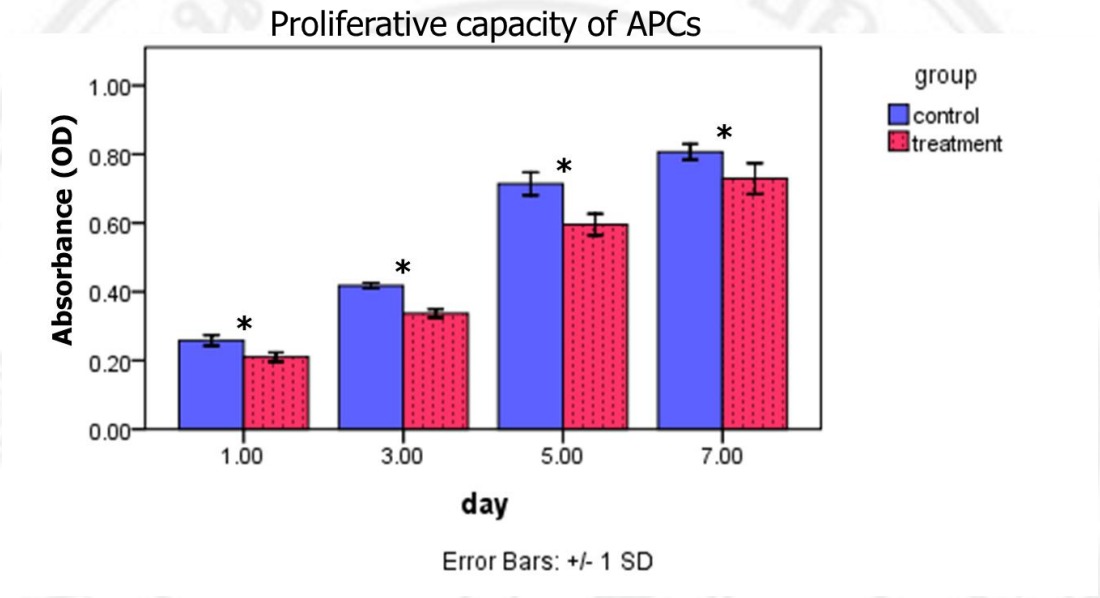

Figure 32: *The proliferative capacity of treated APCs and untreated APCs.* ***.*** *There were statistically significant differences (*; P<0.05) in proliferative capacity of APCs at all time points in the 3Mix-treated group compared to control group (Data were presented in Mean+/-SD).*

3. Mineralization capacity of 3Mix-treated and untreated DPCs/APCs

To determine whether 3Mix-treated DPCs/APCs still had the ability to differentiate into dentin-forming cells, osteogenic/dentinogenic differentiation medium was applied to the cells and the amount of calcium deposits was quantitatively evaluated. All of the samples in the control group and in the 0.39 µg/mL 3Mix-treated group showed positive to mineralization staining, indicating that these cells had the capacity to differentiate into osteogenic/odontogenic cells (Figure 33 and 35). For quantitative analysis using the Alizarin red-S de-staining method, the

amount of mineralized matrix formation in the treated DPCs/APCs was significantly lower than in the untreated cells on days 7, 14, and 21 (Figure 34 and 36).

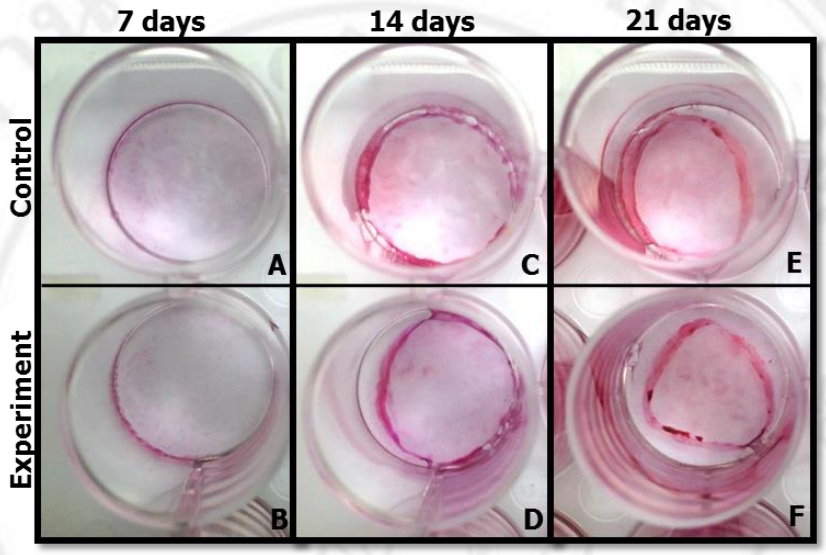

***Figure 33**: Alizerin red-S disclosed DPCs after cultured in differentiating media for 7, 14 and 21 days (from left to right respectively). All of the samples in the control group (A, C, E) and in the 0.39 µg/mL 3Mix-treated group (B, D, F) showed positive to mineralization staining.*

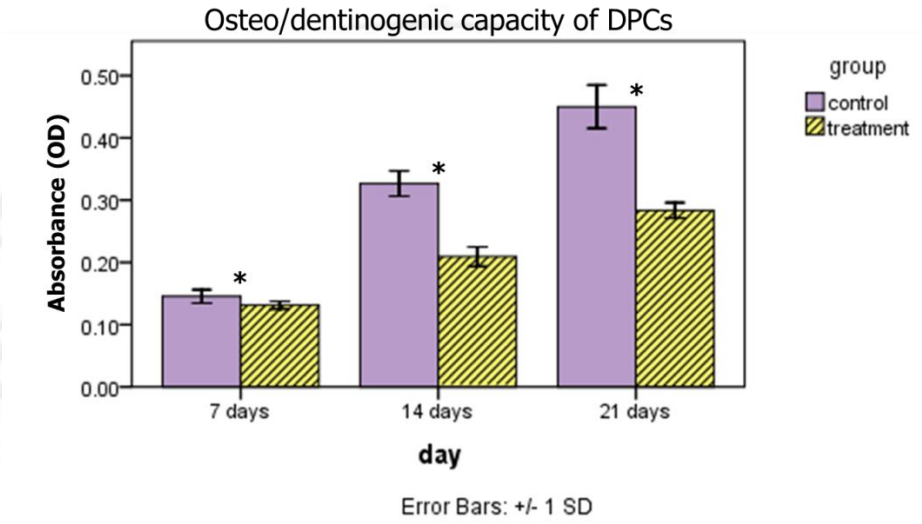

Figure 34: *The quantitative analysis of mineralized matrix formation after treated and untreated DPCs was induced using osteogenic/dentinogenic differentiation media for 7, 14 and 21 days. There were statistically significant differences (*; P<0.01) in odontogenic-osteogenic competency of 3Mix-treated DPCs at these time points.*

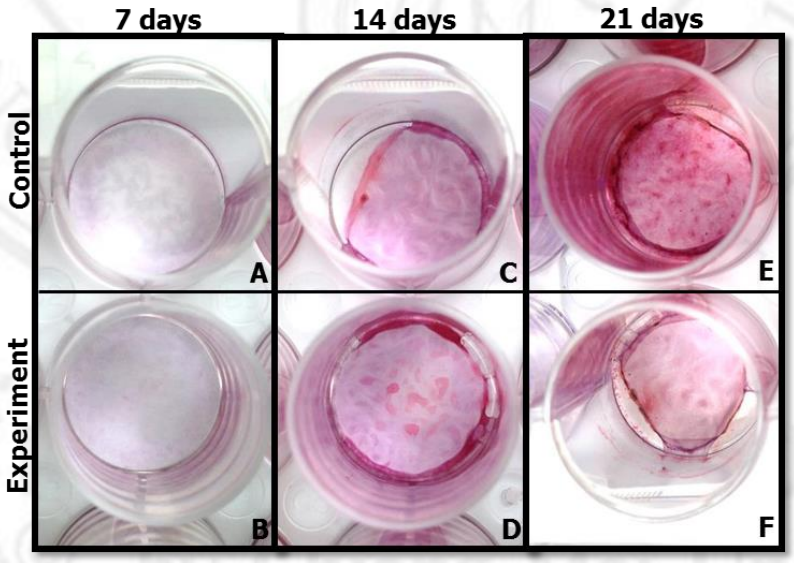

Figure 35*: Alizerin red-S disclosed APCs after cultured in differentiating media for 7 , 14 and 21 days (from left to right respectively). All of the samples in the control group (A, C, E) and in the 0.39 µg/mL 3Mix-treated group (B, D, F) showed positive to mineralization staining.*

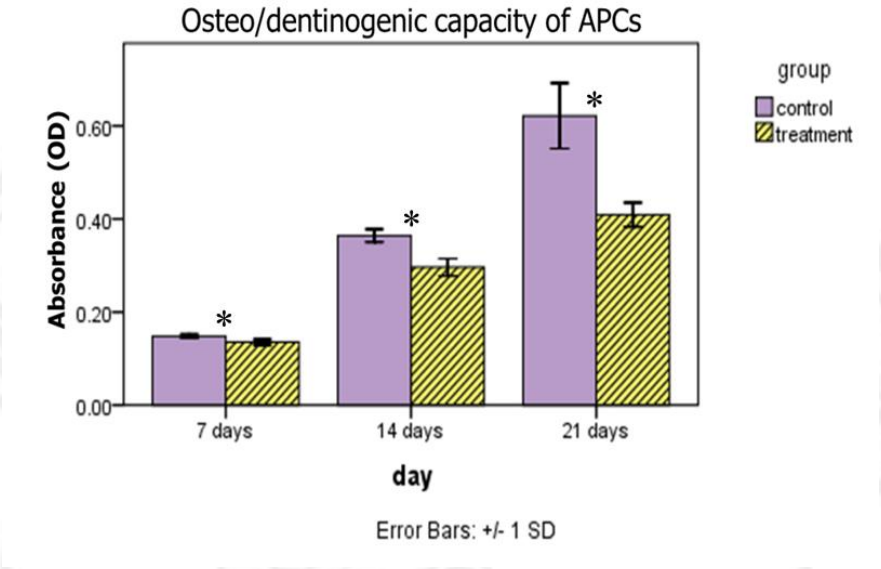

Figure 36: *The quantitative analysis of mineralized matrix formation after treated and untreated APCs was induced using osteogenic/dentinogenic differentiation media for 7, 14 and 21 days. There were statistically significant differences (*; P<0.01) in odontogenic-osteogenic competency of APCs at these time points.*

4. Quantitative Real time RT-PCR

4.1 Gene expression in DPCs compared to treated DPCs

The expression of the BSP gene in DPCs was up-regulated in the 3Mix-treated group compared to the untreated group on days 7 and 14, while ALP and DMP-1 gene expression were lower in the treated group than in the control group, especially on days 14 and 21. However, there was no statistically significant difference (95% CI) in any gene expression between the 3Mix-treated DPC groups and the untreated groups at all time points (Figure 37).

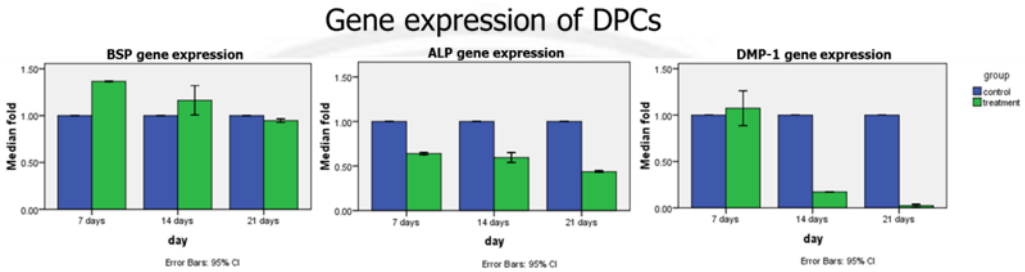

Figure 37: *Charts showing the expression of the BSP, ALP and DMP-1 genes from treated DPCs compared to untreated DPCs. There were no statistically significant differences between 3Mix-treated and untreated DPCs (95%CI).*

4.2 Gene expression in APCs compared to treated APCs

In the APC groups, the expression of the BSP gene was higher in the 3Mix-treated groups than in the untreated groups on days 14 and 21. The DMP-1 and ALP genes were also up-regulated in the treated groups on days 7 and 14, respectively. However, there were down-regulated on day 21. There were no statistically significant differences (95% CI) in gene expression between treated and untreated APCs (Figure 38).

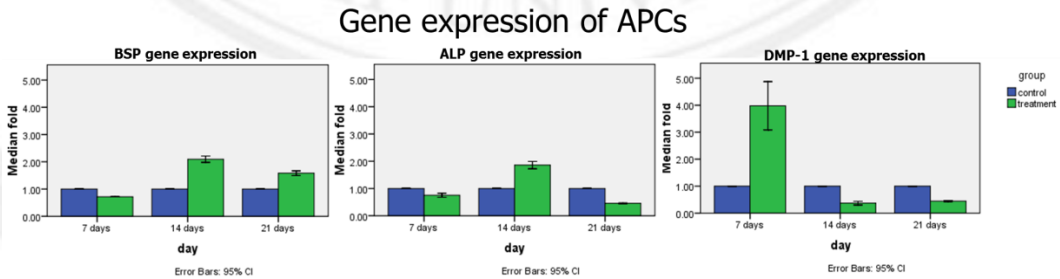

Figure 38: *Charts showing the expression of the BSP, ALP and DMP-1 genes from treated APCs compared to untreated APCs. There were no statistically significant differences between 3Mix-treated and untreated APCs (95%CI).*