CHAPTER 4

Results

1. Morphology of cultured primary human APCs

After enzymatic extraction, APCs were monitored daily under an inverted-light microscope. One day after cell extraction, extracted cells gradually clustered and attached to the culture plate surface.

On day three, cells began to spread out and moved from the digested tissue. Most of the cells had spindle-shaped morphologies with numerous cytoplasmic processes. On the seventh day of the monitoring, more cells were found (Figure 19).

Cells at the third passage were used in the experiment.

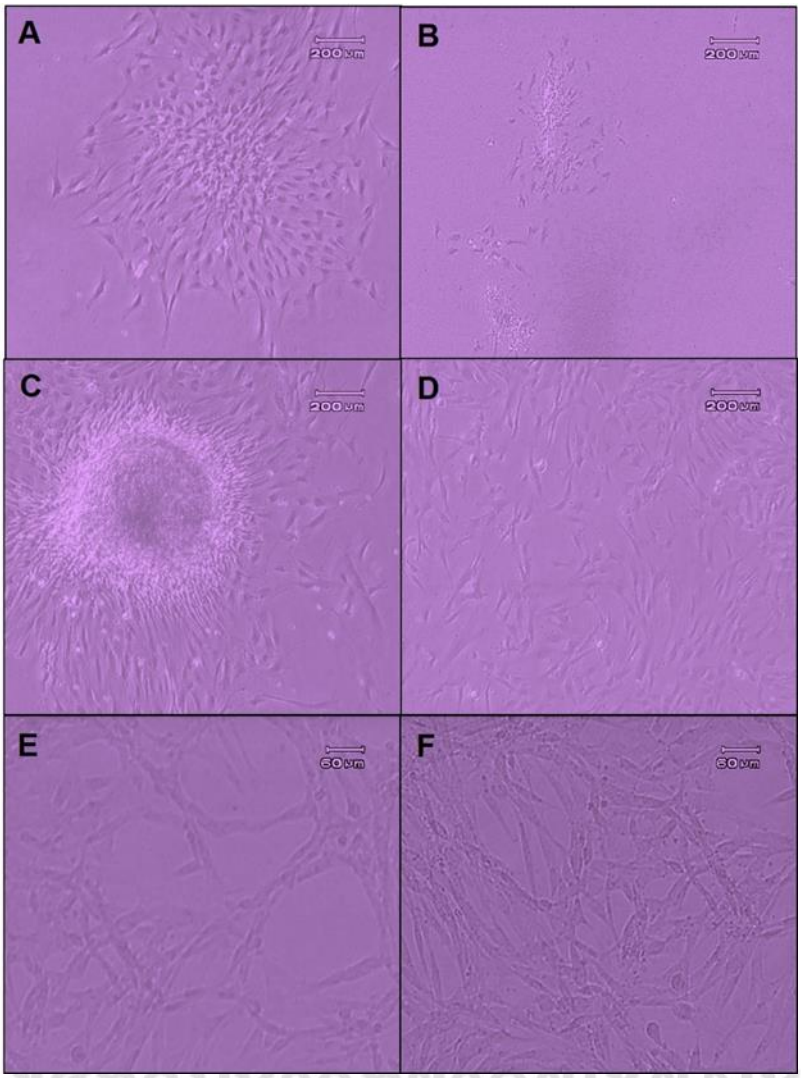

Figure 19 Monitoring of cultured human APCs under an inverted-light microscope. Cells began to spread out and move from the tissue on day three after cell extraction (A and B) (40X magnification). Seven days after cell extraction, more proliferated and attached cells were found (C and D) (40X magnification). At the high magnification of APCs on day seven (E and F), most of cells were spindle-shaped with numerous cytoplasmic processes (60X magnification).

2. Attachment ability and fibronectin expression of APCs to treated dentine slices

The pilot study of fibronectin immunofluorescence was performed to obtain the proper concentration of each reagent. APCs were seeded and stained on sterile glass slides. After immunofluorescent staining of fibronectin, cells on glass slides were observed under a fluorescence microscope.

The nuclei were presented in blue, whereas the expression of fibronectin was presented in green (Figure 20). Cellular fibronectin was obviously present throughout the cytoplasm of the cells.

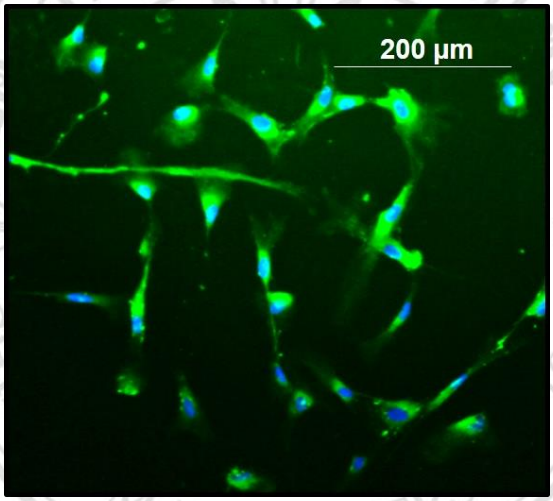

Figure 20 Immunofluorescent staining of APCs on a glass slide. The nuclear marker is presented in blue, whereas fibronectin is presented in green.

After achieving the appropriate concentrations, APCs were seeded on treated dentin slices and the fibronectin positive-staining cells were monitored.

The number of fibronectin-positive cell was lowest in the dentin slices treated with 3Mix paste ($p<0.05$). Pre-treating the dentin with 3Mix at 100 µg/mL or 0.39 µg/mL did not affect the amount of APC attachment compared with the control group (no medication). Dentin treated with $Ca(OH)_2$ at 1 mg/mL or 1,000 mg/mL had significantly higher fibronectin-positive cell attachment than that treated with 3Mix at 100 µg/mL or 3Mix paste (Figure 21, 22)

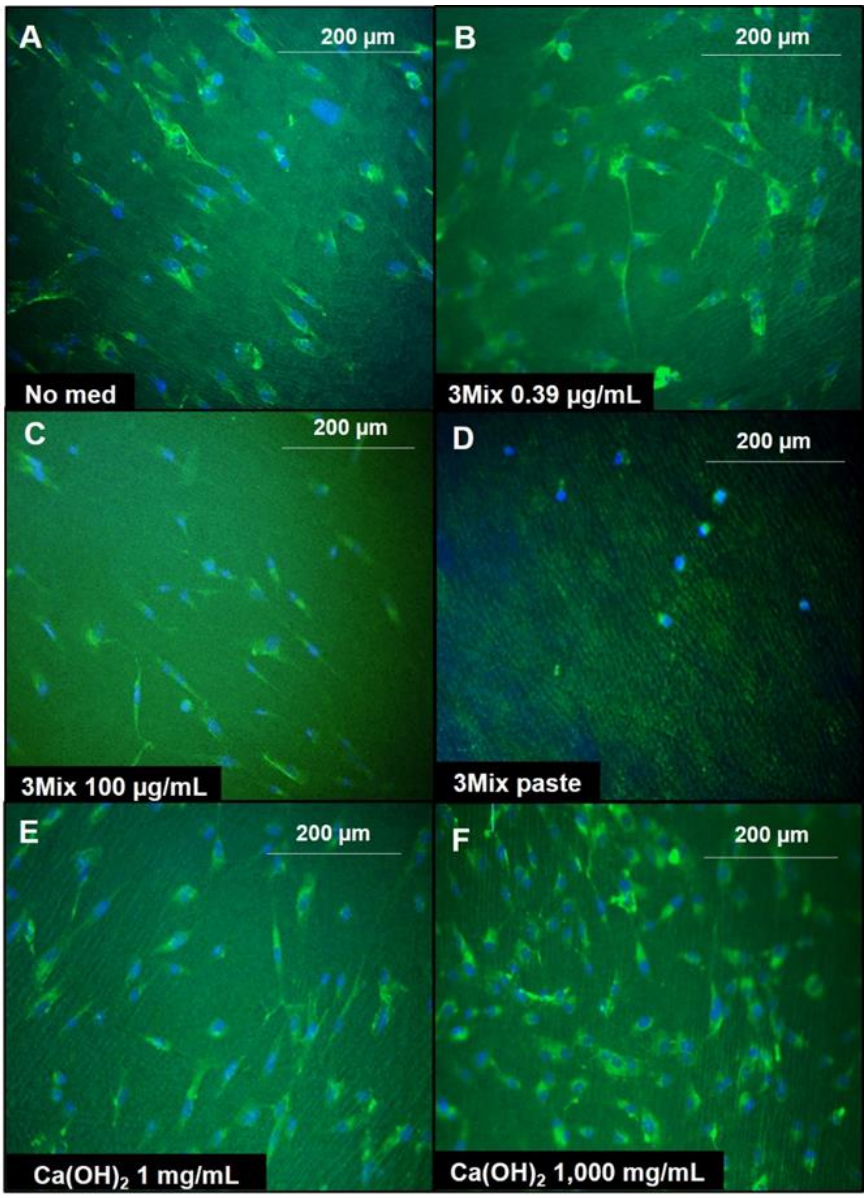

Figure 21 Fibronectin-positive cells attached to treated dentin surfaces. The nuclear marker (DAPI) is visualized in blue, whereas expression of fibronectin is visualized in green. Dentin slices treated with 1,000 mg/mL of $Ca(OH)_2$ (F) presented the highest number of fibronectin-positive cells, whereas dentin slices treated with 3Mix paste presented the lowest number (D).

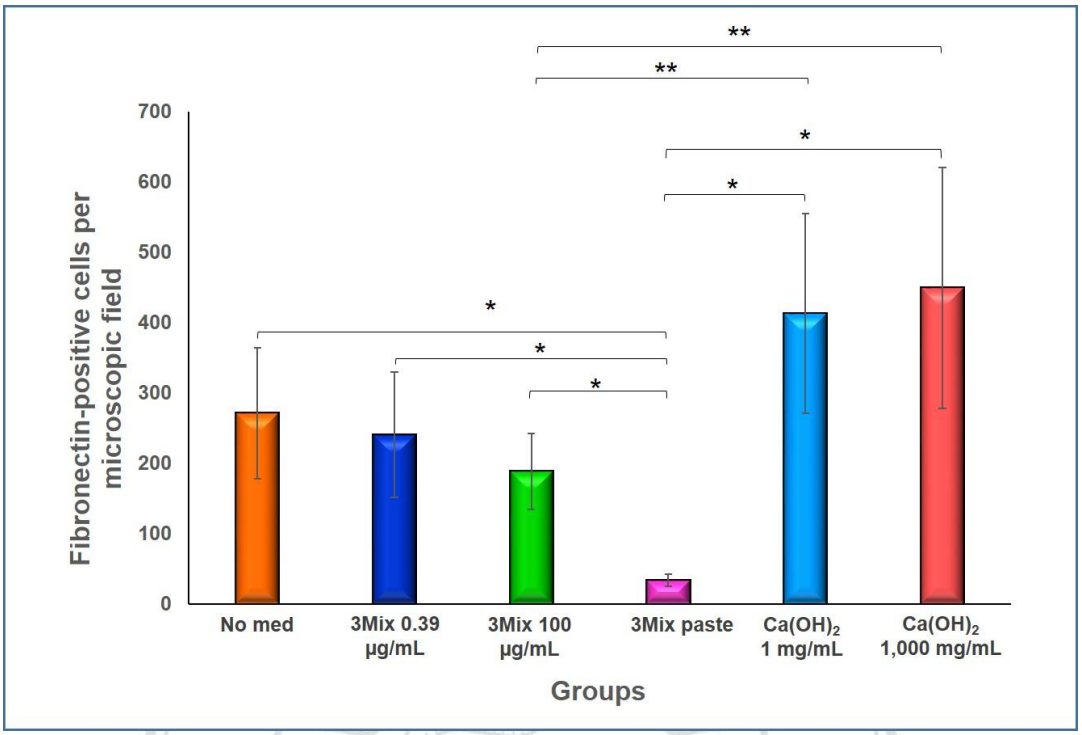

Figure 22 The number of fibronectin-positive cells per microscopic field of treated dentin slices. There were statistically significant differences (*p<.05) in the number of fibronectin-positive cells of 3Mix paste-treated dentin slices compared with the other groups. The number of fibronectin-positive cells on dentin slices treated with 1 mg/mL and 1,000 mg/mL of $Ca(OH)_2$ was significantly higher (**p<.05) than that of cells treated with 100 µg/mL of 3Mix.

3. APC viability monitoring using the alamarBlue® assay

In 3 Mix paste group, the data could not be analyzed since the strong color of 3 Mix, at high concentration, was released into the media disturbing the reading of alamarBlue® assay. APCs on the dentin slices treated with 100 µg/mL of 3Mix had significantly lower cell viability than cells grown on the culture plate (negative control) at all time points ($p<0.05$). Non-medicated dentin slices (no medication group) contained fewer cells than did the negative control. Treatment with 1 mg/mL or 1,000 mg/mL of $Ca(OH)_2$, or with 0.39 µg/mL or 100 µg/mL of 3Mix did not change the viability of APCs on dentin (Figure 23)

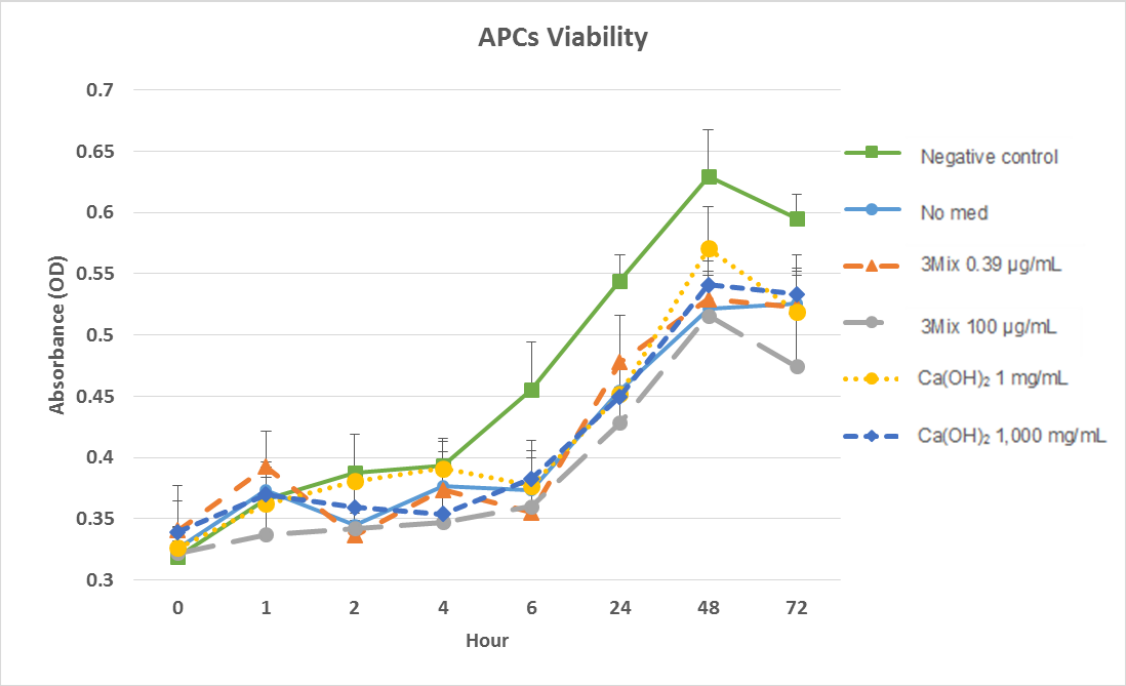

Figure 23: APC viability on treated dentin slices after culture for 72 hours. There were statistically significant differences (p<.05) in the proliferation rate of APCs grown on dentin slices treated with 100 µg/mL of 3Mix at all time points compared with the control group.

4. Scanning Electron Microscopic Observation of APC attachment

The morphology of APCs on dentin slices was observed under a scanning electron microscope at x 2,000 magnification. The cells attached to $Ca(OH)_2$-treated dentin (1mg/mL and 1,000 mg/mL) had spindle-shaped morphologies and evident cytoplasmic processes. On the other hand, APCs attached to the other treated dentin groups had morphologies that ranged from rectangular to round. (Figure 24).

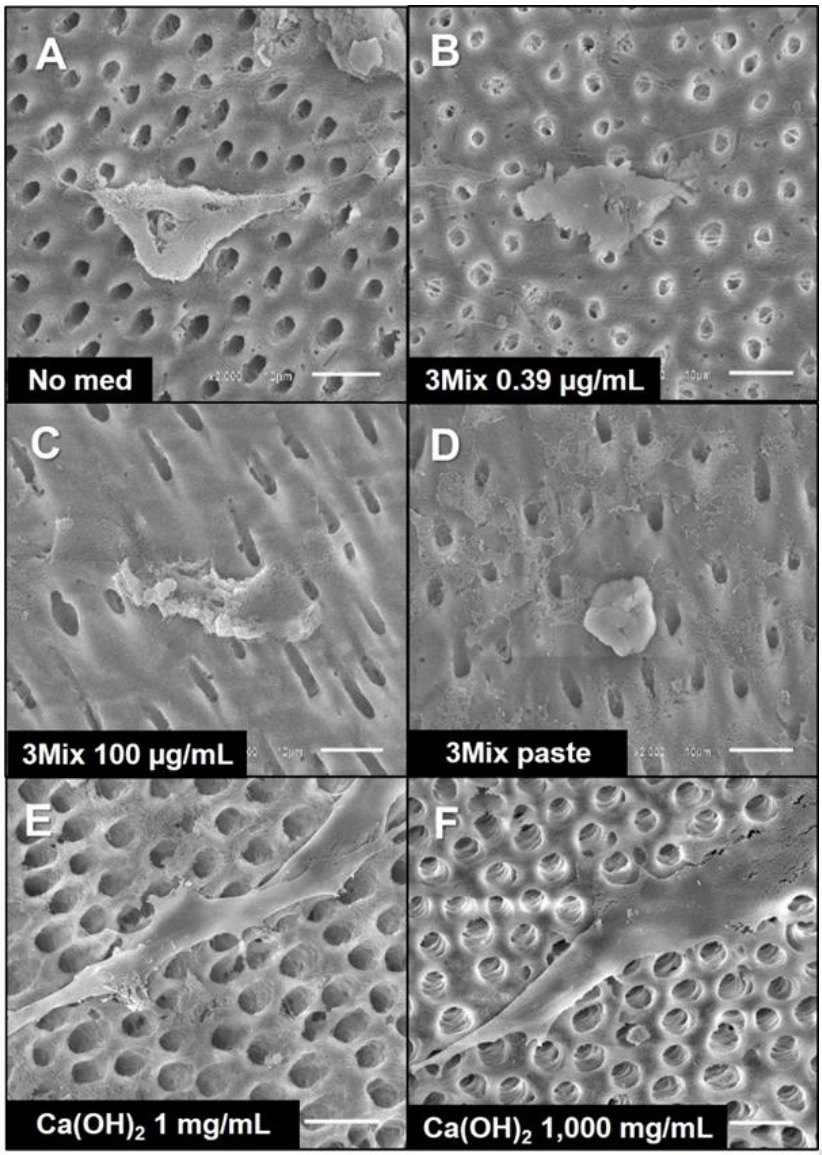

Figure 24 Scanning electron microscopic views of APCs attached to dentin surfaces at x 2,000 magnification. The morphology of APCs attached to $Ca(OH)_2$-treated dentin (E and F) presented with spindleshapes and elongated cytoplasmic processes. The APCs in the other groups (A-D) had short cytoplasmic processes and round morphologies.