CHAPTER 4

Results

4.1 Morphological features of cultured APCs

Twenty-four hours after apical papilla tissues from the individual hosts were digested by specific enzymes, attachment of many clusters of cells on the culture plate surface was observed using an inverted-light microscope. Additionally, at 72 hours' incubation, the cells expanded from their origin tissue. Their appearances were spindle-shaped with several cytoplasmic processes, and their number increased with time. Finally, cells at the third passage were used in the experiment (Figure. 4.1)

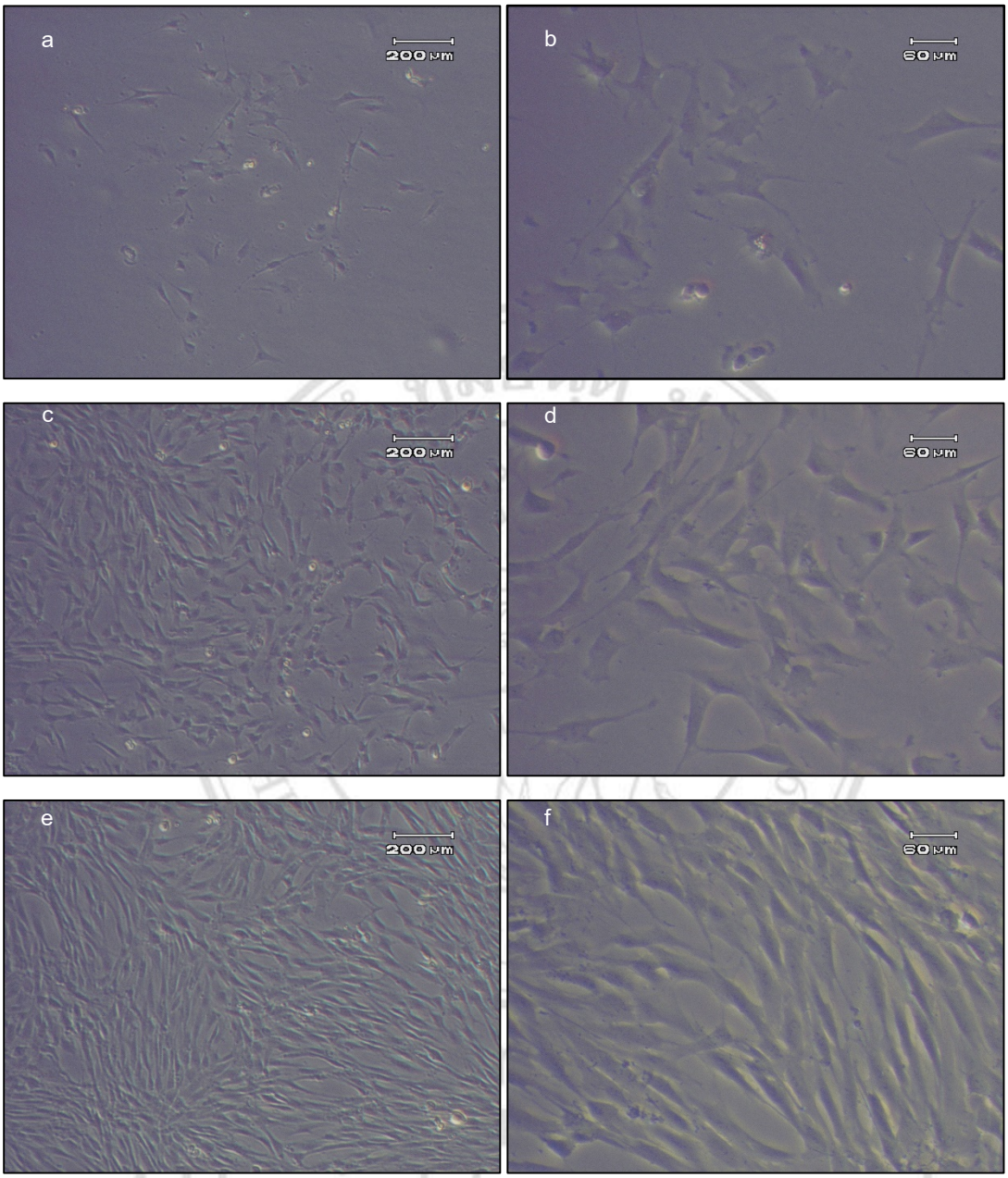


Figure 4.1 Microscopic features of cultured APCs

Observation of morphological appearances of cultured APCs using inverted-light microscopy. After three days, APCs continued to grow and expand their cytoplasmic processes (*a*, at x40; *b*, at x100 magnification). Cells after seven days were gradually proliferating (*c*, at x40; *d*, at x100 magnification). The cells at the 3rd passage in the 75-mL culture flask were used for the attachment assay (*e*, at x40; *f*, at x100 magnification)

4.2 Assessment of APC attachment to previously conditioned dentin slices

Fibronectin-positive cells from three microscopic areas of each sample were randomly counted, averaged, and analyzed as follows:

4.2.1 Overall assessment. The number of fibronectin-positive cells was higher in all dynamic irrigation groups (NI, NI+EA, and NI+PUI) than in the non-dynamic (Control) group ($p<0.001$) (Figure 4.2). Among the dynamic irrigation groups, a significantly greater number of cells were observed when NSS was used as an irrigant than when EDTA, or CHX/EDTA was used ($p<0.001$). No differences in cell attachment ability were observed among irrigating protocols when the three dynamic irrigation techniques were compared. In the control group without dynamic irrigation, a significantly lower number of cells were observed when CHX/EDTA was used ($p<0.01$).

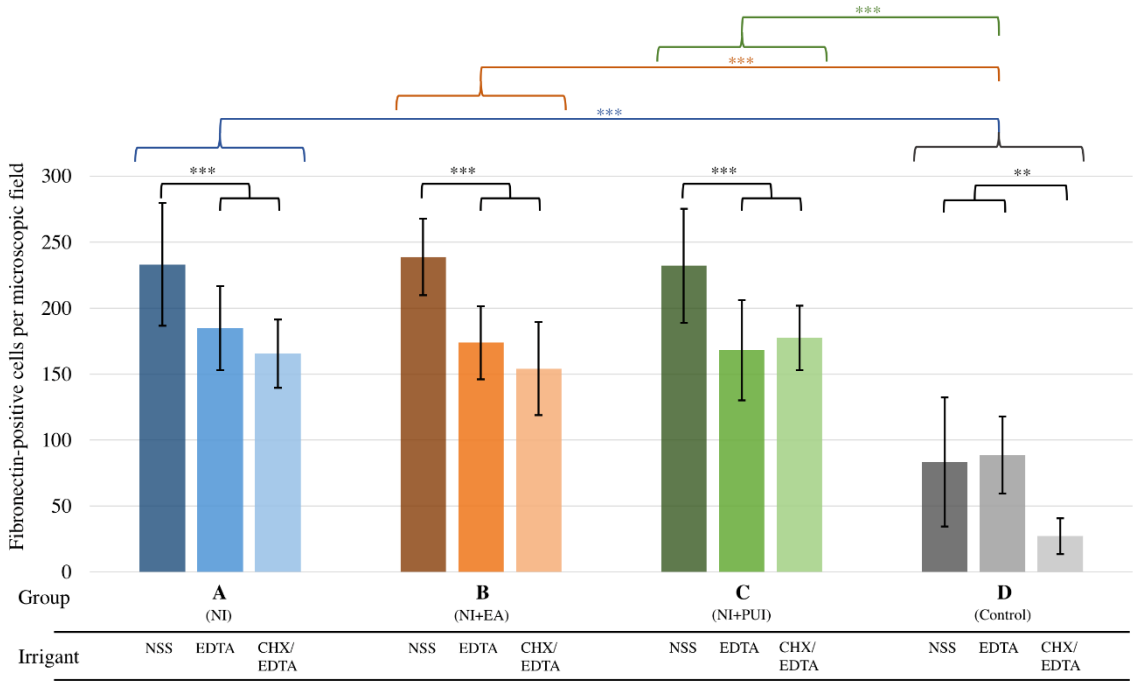
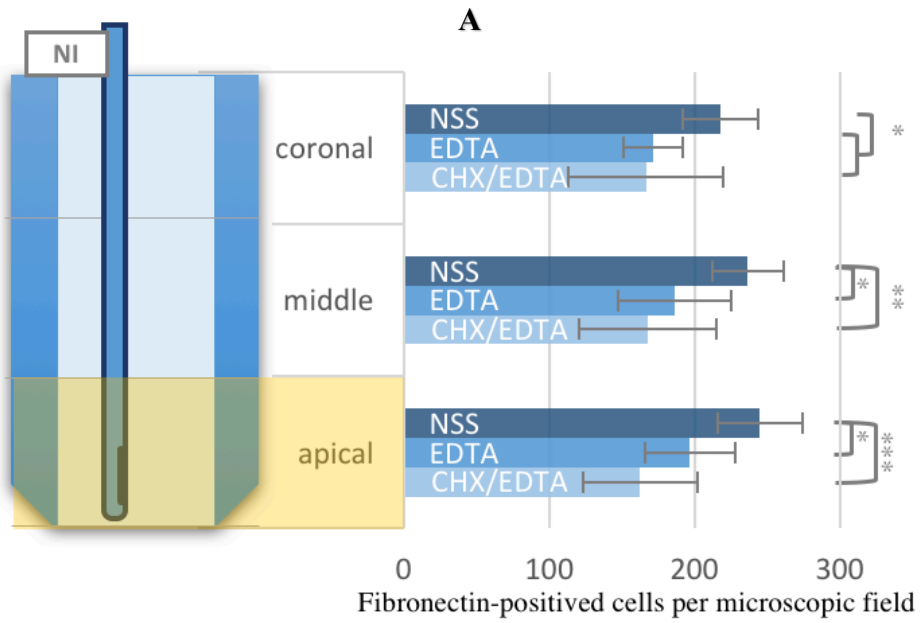


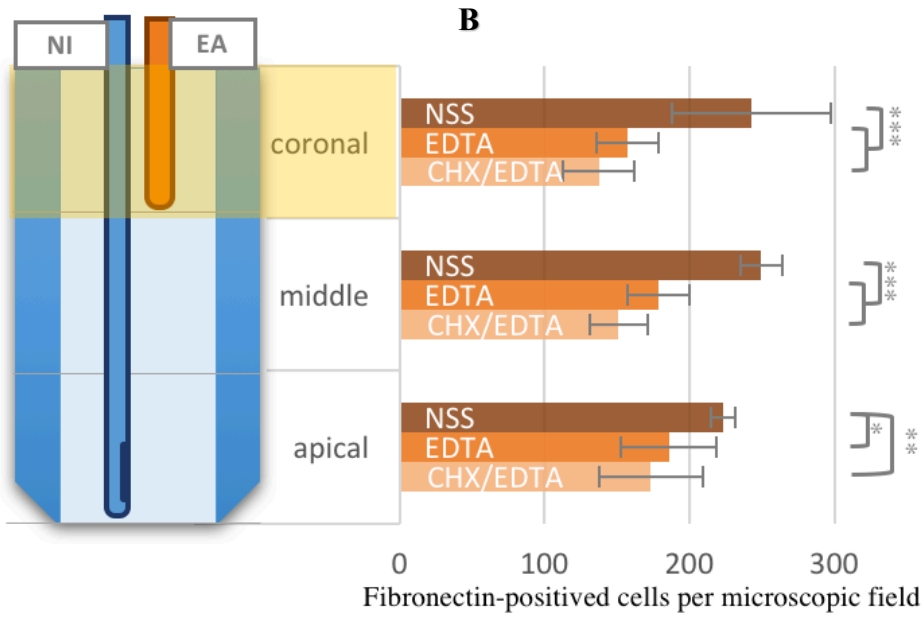
Figure 4.2 Overall assessment of fibronectin-positive APCs on the conditioned dentin of various experimental groups

Overall assessment of fibronectin-positive APC numbers on human root canal dentin after conditioning with various techniques: (*A*) needle irrigation, NI; (*B*) needle irrigation supplemented with sonic irrigation, NI+EA; (*C*) needle irrigation supplemented with ultrasonic irrigation, NI+PUI; and (*D*) control (non-dynamic). All dynamic irrigation groups (*A, B,* and *C*) showed significantly greater cell numbers than the control group (*D*) (***$P < .001$). In each experimental group, a greater number of cells were observed when NSS was used than when EDTA or CHX/EDTA was used (***$P < .001$). In the control group, the least number of cells was detected when CHX/EDTA was used (**$P < .01$).

4.2.2 Multiple level assessment. Since the tip of activated instrument from EA and PUI were intentionally placed at the coronal portion of the root in order to diminish the chance of irrigant extrusion beyond the root apex of the immature tooth, the cell attachment on dentin at three multiple levels: *coronal*, the same level as the tip position; *middle*, the level at 0 – 3 mm beyond the tip; and *apical*, the level at 3 – 6 mm beyond the tip, were separately evaluated.

The number of attached APCs was similar at every level in Groups A (NI) and B (NI+EA). At all levels, when NSS was used as an irrigant, a significantly greater number of attaching cells were found (*$p<0.05$, **$p<0.01$, and ***$p<0.001$) (Figure 4.3*A, B*). In contrast, in Group C (NI+PUI), the cell attachment pattern was not constant. When NSS was used as an irrigant, a significantly greater number of cells were observed at the middle and apical levels (**$p<0.01$ and ***$p<0.001$) (Figure 4.3*C*). A positive trend was observed when PUI was used as a supplement after conventional needle irrigation with NSS. Apart from that, the number of cells was comparable at all levels, regardless of the irrigating solutions used.





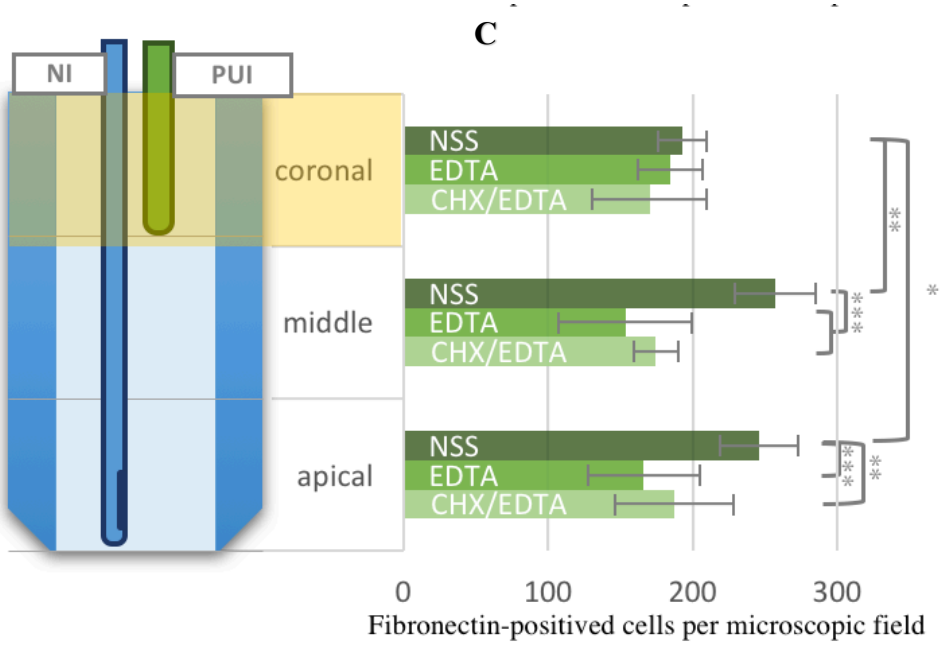


Figure 4.3 Multiple level assessment of fibronectin-positive APCs on the conditioned dentin of various experimental groups

Number of fibronectin-positive APCs on root canal dentin at multiple levels (coronal, middle and apical) after using various irrigation techniques (Groups A, NI; B, NI+EA; C, NI+PUI). In Groups A and B, when the same type of irrigant was used, comparable cell numbers were observed at every level of the root canal. Among irrigants at the same level, NSS significantly increased attached cell numbers. In Group C, the increased cell attachment was significantly greater at the middle and apical levels than at the coronal level, when NSS was used (*$P < .05$, **$P < .01$, and ***$P < .001$).

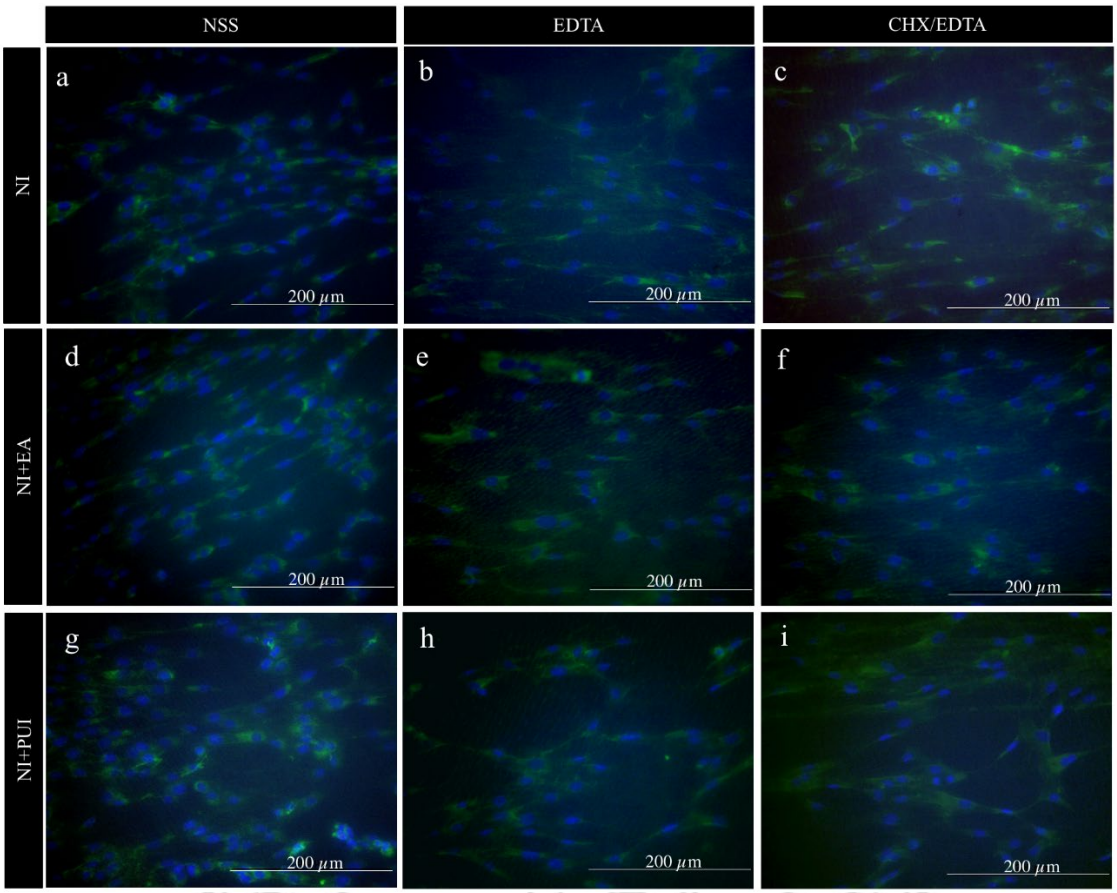


Figure 4.4 Fibronectin immunofluorescent images of cell attachment on the conditioned dentin of various experimental groups

Fluorescent images (at x400) of fibronectin-positive cells from each technique where the tips were safely positioned (as highlighted on the root canals) were categorized as shown (Group A: *a-c*, Group B: *d-f*, Group C: *g-i*). In Groups A and B, samples in which NSS was used (*a, d*), illustrated greater cell numbers than those in which EDTA (*b, e*) or CHX/EDTA (*c, f*) was used. For fluorescent images, the nuclear marker (DAPI) is visualized in *blue*, whereas expression of fibronectin is in *green*

4.3 Dentin morphological evaluation

The dentin surfaces after preconditioning with various dynamic irrigation techniques using a variety of irrigating protocols were qualitatively described. Under 2000x magnification, all dentin specimens had clear surfaces with open dentinal tubules, as shown in Fig. 4.4 (*a* - *i*). Most specimens in which EDTA was used showed more surface cleanliness, as shown in Figure 4.4 (*b, e* and *h*). However, dentin corrosion with collagen exposure was generally observed in the specimens in which PUI was supplemented in the presence of EDTA (Fig 4.4*h*).

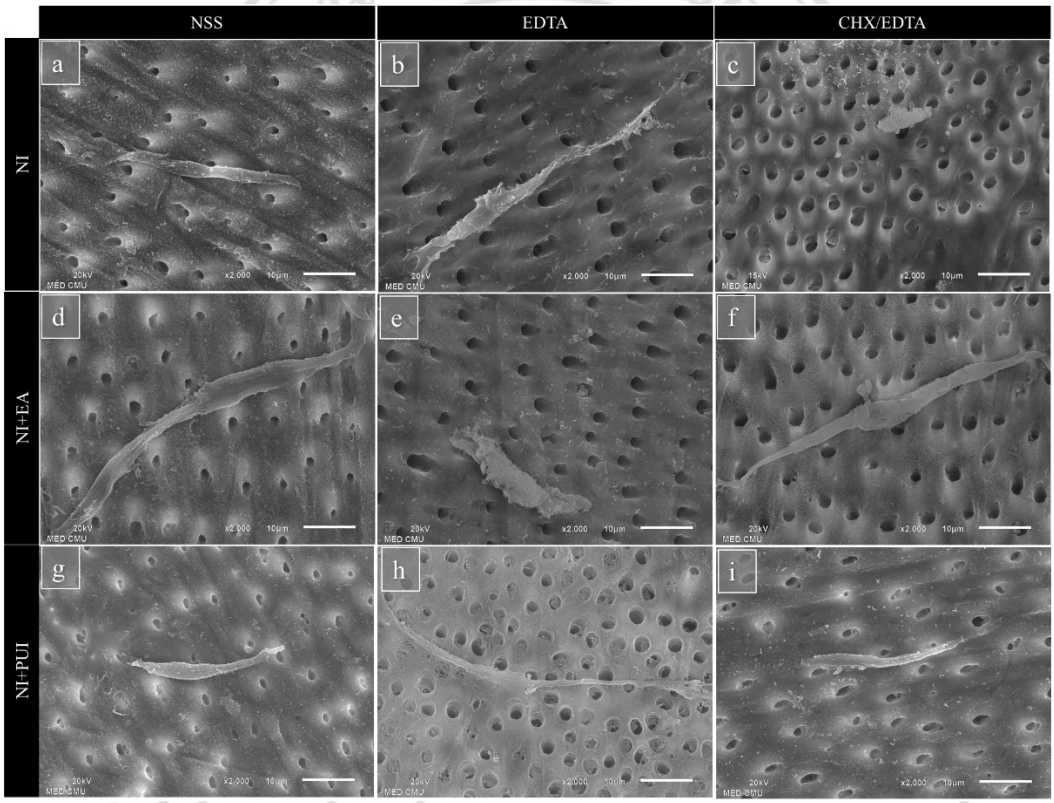


Figure 4.5 Scanning electron microscopic features of dentin morphology of various experimental groups

Scanning electron microscopic images of conditioned dentin samples (x2000). All irrigation techniques exhibited clean dentin surfaces with open dentinal tubules (*a-i*). Dentin corrosion and some collagen exposure were observed only in Group C (NI+PUI) when EDTA was used as an irrigant (*h*).