CHAPTER 5

Discussion

The influence of dynamic irrigation techniques on APC attachment to human root dentin was evaluated in an *ex-vivo* immature tooth model. In this study, either NI, NI supplemented with EA, or NI supplemented with PUI, were applied to the enlarged root canal model along with one of the various irrigating protocols (NSS, EDTA, or CHX/EDTA). A greater number of fibronectin-positive APCs were observed in all specimens in which dynamic irrigation techniques were used than in the group without dynamic irrigation ($p<0.001$). Each dynamic irrigation technique showed similar results in APC attachment. In general, the number of attached APCs was comparable at every level along the root canal, regardless of the dynamic irrigation technique. Nevertheless, among the levels in the group in which NI+PUI was used to activate NSS, significantly fewer attached cells were observed at the coronal level than at the other levels. A significantly greater number of attached cells were observed when NSS was used as a final irrigant ($p<0.001$).

The goals of regenerative endodontics involve not only managing inflammation, but also promoting root continuation by regeneration (75). APCs have been reported to play roles in this regenerative process (5-7, 33). Therefore, to support the proliferation and specific functions of these cells, the root canal system should be cautiously cleaned with an optimal concentration of disinfectants to prevent detrimental effects to the recruited cells (76, 77). Moreover, the dentin surface should be carefully prepared for initial cell attachment in order to promote appropriate tissue generation.

Dentin preconditioning, either by CH medication or EDTA irrigation, has become promising, since positive results showing superior cell attachment have been constantly reported after the use of such interventions (8, 12). Hence, in this study, dentin specimens

were pre-medicated with CH for one week before the experiment, using a previously published protocol (12). After that, all specimens were inserted into the immature tooth model and received the various final irrigation techniques.

The enlarged root canal models were created in this study in order to simulate clinical irrigation procedures for immature teeth that required REPs. Safety should be a concern in the tooth with an open apex, since apical extrusion might not only cause injuries to the patients, but also deteriorate the quality of cells at the apical papilla. In this study, the placement of either an EA or PUI tip as supplementation was limited to the coronal portion of the root, since previous studies reported that apical streaming would flow up to 3 mm beyond the PUI tip (63). However, a side-vented needle was safely placed at the full root length for the needle irrigation as the flow with this needle is confined to the root canal (78). The results revealed that dynamic movement of irrigant was crucial, since the number of attached APCs was greater in every group in which dynamic irrigation was used than in the group with no dynamic irrigation. The increased cell attachment might have been due to the constant refreshing of irrigant, allowing the renewal of active chemical components in solution to work along the root dentin.

EA and PUI agitation generate sonic and ultrasonic energy, respectively, inside the solution. The powerful movement, and the exchange, of irrigants during irrigation efficiently clean the root surface (79, 80). Although many previous studies suggested the effectiveness of either PUI or EA over NI on CH removal (81, 82), it is surprising that in the groups using EA and PUI as supplements in our study, the number of attached cells was not different from the number in the group using only NI. One of the reasons might be that the remaining CH on the root dentin did not really exert negative effects on cell attachment, but, on the other hand, it may have promoted cell survival and cell attachment, as previously reported by some authors (12, 77, 83). Secondly, the effectiveness of both EA and PUI in wide root canals may not be comparable to that in normal root canals. In this study, a 2.5-mm-wide canal was used; therefore, the streaming of sonic and ultrasonic activation may have been less forceful than in normal canals due to the oscillation amplitude, which has been suggested to be 1 mm for EA and 100 μm for PUI (16, 84). Thirdly, the solution may simply have flowed throughout the root canal without the vapor lock effect, since the root canals were already wide and open (85),

making the use of other supplements unnecessary. Thus, any dynamic techniques might have created similar effects, causing comparable cell attachment patterns.

When evaluating cell attachment at different levels, a similar number of cells were observed at every level, either when NI or NI with EA were used. It may be implied that placing EA at the coronal level did not increase the quantity of cell attachment. Placing the tip of the EA at a deeper level in the root canal during activation would also not be suggested, since the cell attachment, even on the dentin at the tip level (coronal), has already shown no better result. Besides, robust extrusion of irrigant would be expected. When considering the use of PUI supplement, the number of cells was significantly higher at the area beyond the tip (middle and apical) when NSS was used. This finding may suggest that placing the tip at the coronal level is suitable enough to promote initial cell attachment inside the root canal, while not causing irrigant extrusion. Therefore, it is suggested that placement of the tips of sonic and ultrasonic equipment should be limited to the coronal part of the root canal.

Regarding the irrigation protocols, the results confirmed that the use of NSS seems to promote better cell attachment in all groups. NSS is a non-toxic solution showing high physiological compatibility, whereas EDTA has higher toxicity but is well-supported for its ability for dentin demineralization and improvement of dentin wettability (15). Many studies have supported the use of EDTA to condition dentin, since increased cell attachment and differentiation have been reported (11, 52). Galler et al have proved significant release of TGF-β when dentin specimens were treated with EDTA or CHX/EDTA (13). The release of TGF-β proportionally increased with time, with 15 minutes showing the highest release. Adjunctive use of 0.12% CHX shortly before EDTA also showed TGF-β1 release, as its acidity improved dentin demineralization. Initially, the author of this study expected that EDTA and CHX/EDTA would enhance APC attachment, but the results clearly demonstrated that the NSS groups showed more attaching cells, regardless of which dynamic irrigation techniques were used. One possible reason might be that the acidity and toxicity of EDTA is still higher than that of NSS (86), so the initial cell attachment is deteriorated. However, the advantage of using EDTA in terms of growth factor release is still very important, since it would stimulate

further cell migration and differentiation (50). Hence, the application of EDTA followed by NSS as a final rinse would be a choice of interest, on which further studies are required.