CHAPTER V

DISCUSSION AND CONCLUSION

Discussion

Various methods have been developed to assess sealing ability of root canal filling materials, usually based on the same principle, i.e., to evaluate the penetration of a tracer along the obturated canal of an extracted tooth. Several tracers, such as dye, radioisotope, and bacterial and their products, had been used for evaluation of microleakage. The dye penetration test is the most popular, probably because it is a simple and inexpensive method. However, this method often yielded a large variation of result, and could hardly be reproducible and comparable (Wu *et al.*, 1993b). Assessment of bacterial leakage might be more biologically relevant than that dye or radioisotope penetration, but the conclusions might vary with the bacterial species used. Maintaining aseptic conditions throughout all steps of the experiment can be difficult. Radioisotope labeling and electrochemical technique were less frequently employed because they pose a radiation hazard and require sophisticated materials and apparatus. The fluid filtration method, which was developed by Derkson *et al.* (1986) for measuring dentin permeability, and later modified by Wu *et al.* (1993a) to evaluation endodontic leakage, was gaining by popularity because it was sensitive nondestructive and permits repeated observation of the same specimen over times. However, there was no standardization of the methods, such as the measurement time, the applied pressure, the diameter of the tube containing the bubble, and the length of the bubble, which might influence the result.

The choice of tracer should be carefully chosen because its size and physiochemical properties may influence the result. The use of tracer of a small molecular size was favored by some authors (Wu and Wesselink, 1993b). The smaller molecular size, and the stricter test, may be seen as more relevant to clinical outcome.

Tray *et al.* (2005a) observed gaps of about 2 µm between the root dentine and the Resilon® primer in a transmission electron micrograph. These imperfections in the bonding might be too small to be detected by bacterial penetration models, as the average length of bacteria varies from 0.2 to more than 10 µm and the width from 0.2 to 1.5 µm. In the present study, glucose was selected as the tracer because it was small molecular size (MW = 180 Da) and is a nutrient for bacteria. If glucose could enter the canal from the oral cavity, bacteria that might survive root canal preparation and obturation could multiply and potentially lead to periapical inflammation. Glucose was thought to be more clinically relevant than other tracers used in microleakage tests. Quantitative analysis of leakage was possible by determining the concentration of glucose in the apical reservoir that leaked through the filled root canal.

Recently, the glucose penetration model, as a new possibility to evaluate the sealing ability of root canal filling, has been introduced described this model as a further development of the fluid transportation concept that might be more sensitive than the measurement with an air bubble (Shemesh *et al.*, 2006).

The model used in this study was modified from that of Xu *et al.* (2005).

The modification was firstly the use of vacuum to reduce air bubbles. Air bubbles could be entrapped in the glucose system and gap between the root canal filling materials and the canal wall. Tracer penetration is affected negatively by air entrapped in the gap between the root canal filling materials and the canal wall, resulting in the failure to demonstrate the full extent of the void. The determination of leakage under vacuum conditions seems to be able to solve this problem. Several studies (Goldman *et al.*, 1989; Oliver *et al.*, 1991; Spangberg *et al.*, 1989; Spradling *et al.*, 1982) have shown a more complete and consistent dye penetration when entrapped air was removed by vacuum. However some studies (Masters *et al.*, 1995; Roda *et al.*, 1995) showed no differences in leakage between vacuum and non-vacuum methods. This controversial might be caused by root position during dye immersion as shown in the study by Katz *et al.* (1998).

Although the use of pressure may have no clinical relevance, it has the practical advantage of accelerating leakage detection.

Secondly instead of using epoxy resin or sticky wax, the contact between tooth and glass tube was sealed with silicone. This was proved to provide better seal in moist environment (95% humidity).

Finally specimens were placed in closed system at 37°C 95% humidity. From the pilot study, this model would eliminate the effect of glucose evaporation on glucose concentration measurements within 28 days.

In positive control group, glucose leakage concentration increased gradually in the first week. In the second week, glucose leakage concentration was rapidly increasing and then became stable at the third week. However, glucose leakage concentration could not be detected in all negative control groups. The experimental groups could be detected glucose leakage concentration. Thus, it should be noted that the concentration value of glucose leakage was lower than previous studies according to the distance differences of the root canal fillings. In this study the root canals were filled up to coronal third as compare with the 4 mm fill of apex in other studies (Shemesh *et al.*, 2006; Xu *et al.*, 2005). The dentinal tubules configuration which was less dense in the apical part than the coronal part (Fogel *et al.*, 1988) might lead to compromised bonding apically.

In this study the root canals were irrigated with 2.5% sodium hypochlorite and 17% EDTA before final flush with sterile water (group 1), 2% chlorhexidine (group 2) and 2.5% sodium hypochlorite followed by 2% chlorhexidine (group 3). There were no significant differences of glucose leakage concentration measured at 28 days in all experimental groups. However, the glucose leakage concentrations in all experimental groups were found significantly increasing from the beginning to the end of experimental period.

In the experimental groups there were combinations of irrigants which resulted in color change and precipitation. Combination of 2% chlorhexidine and 17%EDTA (group 2) demonstrated the formation of a white viscous solution and combination of

2.5% sodium hypochlorite and 2% chlorhexidine (group 3) demonstrated the formation of the dark-brown precipitation.

In group 3, there was more glucose leakage concentration at the end of experimental period when compared with group 2. The dark-brown precipitation occurred when sodium hypochlorite and chlorhexidine were combined might interfere with the sealing of root canal filling therefore occurred gaps between Epiphany® sealer and dentin. In cross-sections of filled roots, gaps were observed between the dentin and Epiphany® layer (Tay *et al.*, 2005a).

Recent studies (Gomes *et al.*, 2002; Zehnder, 2006) have reported the occurrence of color change and precipitation, when sodium hypochlorite and chlorhexidine are combined. Further more concern has been raised that the color change might have some clinical relevance (Gomes *et al.*, 2002) because of staining, and that the precipitation might interfere with the sealing of root canal filling.

Basrani *et al.* (2007) demonstrated the precipitate that sodium hypochlorite mixed with chlorhexidine contains a significant amount of para-chloroaniline (PCA); a hydrolysis product of chlorhexidine and the amount of PCA directly increased with the increment in the concentration of sodium hypochlorite. As the concentration increased the color darkened and the precipitate thickened. Para-chloroaniline (PCA) has industrial uses in pesticides and dyes (Loe, 1973) and has been demonstrated to be carcinogenic in animals (Okino *et al.*, 2004). Its degradation product, 1-chloro-4-nitrobenzene, is also a carcinogen.

Bui *et al.* (2008) compared the effect of irrigating root canals with a combination of sodium hypochlorite and chlorhexidine on root dentin and dentinal tubules. Their findings showed that there were significantly fewer patent tubules in the experimental groups when compared with the negative control group. Because of the sodium hypochlorite/chlorhexidine precipitate tends to occlude the dentinal tubules. Also, the presence of this precipitate on the root surface might affect the seal of an obturated root canal, especially with resin sealers in which a hybrid layer is required.

In group 2, there was less glucose leakage concentration at the end of experimental period when compared with other experimental groups. The white viscous solution occurred when EDTA and chlorhexidine were combined. To date, no studies have demonstrated chemical properties of the white viscous solution or the effect on root dentin. Unpublished data showed final irrigation with 2% chlorhexidine after irrigated with 17% EDTA had tendency increased bond strength of root canal filled with Epiphany® and Resilon®. However, the reason supported this data was not elucidated. Marending *et al.* (2007) proposed an irrigation regimen in which sodium hypochlorite would be used throughout instrumentation followed by EDTA, and chlorhexidine would be used as a final irrigant.

In group 1, the final irrigant was sterile water as recommended by manufacturer of Epiphany® sealer. Although, there was no precipitate and color change but the result showed glucose leakage concentration and the rate of the leakage was gradually increasing in the same pattern as in group 2. The cause of glucose leakage might be explained firstly from the volumetric shrinkage that occurs concurrently with polymerization of the resin. If the resin-tooth bond is too weak, polymerization forces will debond the resin from the tooth and microleakage will result (Kidd, 1976). Secondly during experiment, the root specimens were immerged in the apical reservoir, therefore dissolution of sealer might occur and permit gaps formation between root canal dentin and root filling materials that result in increased bacterial leakage over time. Versiani *et al.* (2006) measured solubility of Epiphany® sealer did not conform to ANSI/ADA standardization (3.14%). Thus, water sorption and solubility play an important role concerning the increased microleakage in long term observation.

In the first week, the rate of glucose leakage was nearly same increasing in all experimental groups. But in the second week, rate of glucose leakage was gradually increasing especially the group 3 which there was more increasing as compared with other experimental groups and became stable after second week until the end of the experiment. Although there were no significantly difference.

Under condition of this study, group 2 was the least glucose leakage concentration that occur the formation of a white viscous solution. Further research needs to study chemical properties of the white viscous mixture of 17% EDTA and 2% chlorhexidine and its effect on root dentin.

Conclusion

There were no statistically significant difference of glucose leakage in root canals filled with Epiphany® and Resilon® after final irrigation with sterile water, 2% chlorhexidine, or 2.5% sodium hypochlorite followed by 2% chlorhexidine after irrigated with 17% EDTA. Thus, this study showed that 2% chlorhexidine when used after 17% EDTA and 2.5% sodium hypochlorite did not adversely affect the leakage of root canal system filled with Epiphany® and Resilon®.