# CHAPTER V

# DISCUSSION AND CONCLUSION

When nonsurgical treatment fails, surgical endodontic treatment is often indicated. A root-end filling is normally placed in conjunction with root-end resection. The commonly used root-end filling material is MTA but flowable resin composite has also been used as an alternative retrofilling material to MTA. One requirement of root-end filling materials is their biocompatibility. This is the first to study evaluate and compare cytotoxicity between flowable resin composite and MTA. Cultured human periodontal ligament cell which is the major cells for wound healing after root end resection was used to evaluate response to four root-end filling materials. The method particularly used to evaluate the cytotoxicity were MTT (functional) and morphological assays. These two methods allow ability to detect the effect of dental materials on cell viability by alterations of either mitochondrial dehydrogenase activities or cell morphology and attachment after in contact with material (Schweikl and Schmalz 1996). Another advantage of this study is the use of both material elution and direct contact on material surface to be investigated. Therefore the cytotoxic effect that are distant from and in contact with material can be observed. The use of elution would simulate the immediate postsurgical root end environment in which toxic element of the retrofilling material leach into the surrounding fluids in bony crypt (Keiser et al. 2000). On the other hand, the direct contact testing would simulate the situation after healing process has occurred and periodontal ligament cells are in direct contact to retrofilling material.

ISO standard 10993 calls for the eluates of the dental materials for cytotoxicity testing to be prepared so that the ratio of the surface area of the test material to the volume of the extraction vehicle is to be between 50.0 and 600 $mm^2$/ml (Keiser et al. 2000). In the present study the surface area to volume ratio used for eluate preparation was 425 $mm^2$/ml conforming to ISO standards allows better comparison of results between different studies.

In this present investigation, after materials were immersed in cell culture medium for 1, 2, 3 or 4 days, extracted medium from Tetric® Flow, Aeliteflo™ and MTA showed higher percentage of cell viability beyond control. This may be due to the variation that could occur in this type of experiment; however, this difference is not statistically significant. On the other hand, extracted medium from Filtek™ Flow was cytotoxic at 1 and 2 days when compared with control. When Filtek™ Flow was immersed for longer period, cytotoxicity appeared to be washed out and showed no cytotoxicity compared to control. This could be inferred that extracted medium withdrawn from flowable resin composites was low toxicity and not different from MTA throughout the experiment. The absence of toxic in flowable resin composite from this study is in agreement with Nalcaci and co-worker (2004). Their study demonstrated that eluates from flowable composite polymerized with standard cure procedure showed no toxicity (95.5% cell survival rate) eventhough the material was aged for only one day. The method in that study was similar to our study except the type of cell and brand of flowable resin composite. They used L929 mouse fibroblast to test with Flowline® which was flowable resin composite that contained TEGDMA and methacrylate in resin matrix. While in our study, Tetric® Flow containing Bis-GMA, UDMA, TEGDMA, Filtek™ Flow and Aeliteflo™ containing Bis-GMA and TEGDMA were used to test with

human periodontal ligament cells. However, study of Wataha and coworker's (2003) found that cytotoxicity of leachable from Tetric® Flow was very toxic (<50% survival rate). This may be due to different extraction media used. In their study, they used artificial saliva to elute monomer substance from resin composite. But in our study, we used culture medium with 10% FCS as extraction media. Moharamzadeh and colleague (2007) tested different extraction media on monomer release from resin composites. They detected that quantity of monomer extracted with artificial saliva was higher than that with culture media. The type of extraction medium may have a significant effect on monomer release from resin composites.

When the viability of cells in direct contact with materials was examined, all freshly mixed materials revealed cytotoxicity effect to HPDLs. Tetric® Flow, at this state, was the least toxic compared to other flowable resin composites and MTA. After material was immersed in cultured medium, toxic substances seemed to be removed. Therefore, residual monomers that left in resin composite may be fewer. It is known that these residual unpolymerized monomers in resin composites are cellular toxic. However, in this study these unpolymerized monomers in oxygen-inhibited layer were first removed from material by 70% alcohol (as recommended by Rud et al (1991a)) and the resin composite were also eluted using culture medium. This rationale was supported by Mohsen and associates (1998). They found that removing the oxygen-inhibiting layer from the resin composite decreased the cytotoxicity by 33%. Untreated resin composite samples showed the most cytotoxicity while samples that were eluted in water or alcohol had lower toxicity. Alcohol was proved to be better extraction medium than water in that study. This was also supported by Ferracane's study which found that 50% (by weight) of the

leachable species eluted from resin composites within 3 hour after immersion in water. But when material was immersed in ethanol, 75% of these molecules were eluted. After 3 hours, elution was slower and essentially complete after 24 hour (Ferracane 1990). Similar finding was also revealed by Vahid and colleagues. The major reduction of monocyte viability in that study was observed after 36 hours exposure and this may indicate that the main part of monomers are released during early hours after polymerization. After that only few monomer was released or left in resin composite (Vahid 2004). Different resin composite with different composition may require different time to complete its monomer leaching. This might be the reason that Tetric® Flow and Filtek™ Flow became non toxic sooner than Aeliteflo™.

Extract medium from freshly mixed MTA in this study demonstrated high toxicity (thirty six percent survival rate). The similar result was also found in previous study using human periodontal ligament cell. They discovered low cell viability in freshly mixed MTA which was 40% approximately (Keiser et al. 2000). But the result was in contrast to Torabinejad et al. (1995c) who reported low cytotoxicity in freshly mixed MTA. This may be due to differences in experimental procedures and cells used. Torabinejad et al (1995c) used mouse fibroblasts and an agar overlayer method that requires diffusion of toxic components of the material over time. While in this present study, we used a primary cell line and eluates of the materials that allowed a continuous contact with the cells used.

Material evaluation from scanning electron microscope revealed the difference in surface roughness between flowable resin composites and MTA. Surface of MTA appeared to be rough and it composed of larger particles

while flowable resin composites consist of fine filler particle appeared smoother. The morphology assay of cells assessed by scanning electron microscope also indicates that HPDLs attached and spreaded well on Tetric® Flow and MTA. HPDLs spreaded on Filtek™ Flow but not as well as Tetric® Flow and MTA did. On the other hand, cells did not spread but appeared rounded with small process in the presence of Aeliteflo™. The results of this study agree with previous *in vitro* study that found a favorable response of osteoblasts to MTA and resin composite (Z100). In that study, materials were also allowed to set and its cytotoxicity was eluted using culture medium (Zhu et al. 2000). *In vivo* studies of Torabinejad and colleagues (Torabinejad et al. 1995a) and Andreasen and colleagues (Andreasen et al. 1993) were also in agreement to this study. They found cementum formation on the surface of MTA and resin composite (Retroplast). However, in Haglund and colleagues' study (Haglund et al 2003), they found that set MTA had no effect on cell morphology while set Retroplast resulted in irregular appearance of L929 cells. This may be due to the surface of resin composite samples in the study were neither polished nor wiped with alcohol pellet, leaving uncured oxygen-inhibited layer remained on its surface and caused toxicity to cells.

There were also some limitations in the present study. As this was an *in vitro* experiment using cultured human periodontal ligament cells, the results obtained from this trial can only assess the cytotoxicity of flowable resin composites and MTA to cultured human periodontal ligament cells. The whole mechanisms of postsurgical healing process *in vivo* are more complex, involved both cellular and extracellular events. Due to the nature of experiment, the number of samples was limited. Thus, the results cannot be completely judged to total populations. Further *in vivo* and clinical investigations are

needed before this material can be recommended for routine use in clinical practice.

In conclusion, flowable resin composites showed low level of cytotoxicity in both elution and direct contact in comparable to MTA. They also demonstrated good attachment of cells when contacting to materials. Tetric® Flow, in particular, revealed less toxicity than Filtek™ Flow in elution. It is also more biocompatible than Aeliteflo™ in direct contact testing and scanning electron microscope observation. Therefore flowable resin composite might be eventually considered as alternative retrofilling material to MTA.