CHAPTER V

DISCUSSION AND CONCLUSION

Our study revealed a new finding of the effect of dental radiation in the mGy range on primary human bone cell. The results indicated that dental radiation, the lowest diagnostic dose that would not be expected to have any influence on any cell type behaviour, was not toxic to primary bone cells as determined by MTT assay. In contrast, we observed a slight increase in the assay. Whether the increase was due to the higher metabolism of cells or to the increase number of cells requires further investigation. However, our result was in concurrent with others showing that low dose radiation (< 0.05 Gy) could induce cell proliferation in a dose dependent manner in various cell types such as human lung fibroblasts, normal human diploid cells, Chinese hamster fibroblasts, neuron cells, and hematopoietic cells.(Kang *et al.*, 2006; Kim *et al.*, 2007; Korystov *et al.*, 1996; Wang and Cai, 2000; Watanabe *et al.*, 2002) Many studies suggest that the stimulation of cellular proliferation by low dose radiation involve the activation of growth factor receptors in the plasma membrane (Dent *et al.*, 1999; Goldkorn *et al.*, 1997) However, the exact mechanism of low dose radiation-induced bone cell proliferation is still unclear.

The downregulation of bax pro-apoptotic gene might involved p53 protein, a tumor suppressor gene product whose function is involved in cell cycle arrest, apoptosis, DNA repair, and senescence following irradiation.(Chendil *et al.*, 2000; Herzog *et al.*, 2007; Kastan *et al.*, 1995) It is widely accepted that the bax promoter contains a typical p53 tumor suppressor gene-binding site and that p53 exerts its role as inducer of apoptosis partly by trans-activating the expression of bax gene.

(Miyashita and Reed, 1995) It has been shown that p53 upregulated the synthesis of Bax while downregulated that of Bcl-2 (Karpinich *et al.*, 2002; Weller, 1998) A number of studies illustrate that p53 mediates the apoptotic response following irradiation related to the ability of p53 to regulate the expression of pro- and anti-apoptotic members of the Bcl-2 family (Miyashita *et al.*, 1994). Interestingly, p53 accumulation was not observed after irradiation with low dose radiation (<0.5 Gy). (Kim *et al.*, 2007; Watanabe *et al.*, 2002)Thus, it is possible that the mGy range dental radiation used in this study had no effect on p53 activation, as a result, the downregulation of bax expression was observed.

Radiation-induced apoptosis is a caspase-dependent mechanism. (Bacqueville and Mavon, 2008; Yamakawa *et al.*, 2008) Among the effector caspases, caspase-3 plays an important role in the apoptotic process. The reduction of caspase-3 mRNA expression following dental irradiation in our study indicated that dental radiation could reduce the apoptotic cell death, perhaps, due to the significant decrease of BAX/BCL-2 ratio when irradiation with 2 doses of dental radiation. The previous study showed the reduction of caspase-3 activation was observed when the level of p53 was low.(Katiyar *et al.*, 2005) Thus, the reduction of caspase-3 expression in our study may involve the low accumulation of p53. However, the examination of p53 expression and/or activation is needed to clarify this hypothesis.

Another important apoptotic indicator is the Bax/Bcl-2 ratio. The decreased ratio of Bax/Bcl2 in a given cell determines its susceptibility to apoptosis. (Kaseta *et al.*, 2008; Yin *et al.*, 1994) It has been suggested that the Bax/Bcl-2 ratio may be more important than either promoter alone in determining apoptosis. (Stoetzer *et al.*,

1996) Although, in this study, the expression of both Bcl-2 and Bax tended to decrease after irradiation, only the reduction of Bax was statistically significant, resulting in the decreased of Bax/Bcl-2 ratio after irradiation. In the case of high expression of Bcl-XL protein, it has been reported that the Bax/Bcl-2 ratio might not be used as apoptotic indicator (Lee *et al.*, 1999) To clarify the hypothesis, we have investigated the level of Bcl-xl expression. However, no significantly changes in Bcl-xl expression was observed. In addition, Salakou et al. revealed that the increased Bax/Bcl-2 ratio upregulated caspase-3 and increased apoptosis (Salakou *et al.*, 2007) . Thus, the reduction of both Bax/Bcl-2 ratio and caspase-3 expression indicated that dental radiation could decrease bone cell apoptosis possibly by the regulation of bcl-2 family proteins.

Interestingly, the effect of dental diagnostic radiation could be differently observed on different primary bone culture. All bone cultures illustrated the changes on apoptotic-related genes following 4 hours irradiation. Most of the preparations showed the downstream in both pro-apoptotic and anti-apoptotic genes, while the others showed conversely. The difference in the response to low dose radiation may be due to the genotypic difference of the doners or difference in the osteoblastic stages of differentiation. Recent studies proposed that radiosensitivity may be a genotype-dependent (Lindsay *et al.*, 2007; Williams *et al.*, 2008) since the clonogenic survival, radiation-induced apoptosis and radiation-induced redistribution in the cell-cycle vary in the different cell line.

In conclusion, dental radiation could induce apoptotic-related gene expression both anti-apoptotic group and pro-apoptotic group. The response to low dose radiation

might be a genotype-dependent. Thus, prescribing dental radiographs should be highly selective based on the principle of ALARA (As Low As Reasonably Achievable).