CHAPTER I

INTRODUCTION

Periapical radiograph is a common radiographic technique used in dental clinics because it provides several advantages among the other dental imaging modalities, such as the least distortion, the highest resolution and the lowest radiation dose (Vivek and Byron, 2000). Exposure to ionizing radiation even 1 milligray caused DNA double-strand break of fibroblast cells (Rothkamm and Lobrich, 2003) which determined critical molecular lesion leading to cell death (Dugle *et al.*, 1976; Hoeijmakers, 2001a; Radford, 1985; Radford, 1986). The third quartile patient entrance dose for intra-oral radiograph is 3.9 milligray (mGy) (Napier, 1999). Thus, dental radiation might cause DNA lesion of oral cells leading to cell death through apoptotic process. This hypothesis was supported by Branemark and associates (Branemark *et al.*, 1990) who did not recommend performing dental radiographic procedures immediately after implantation due to the possibility of the detrimental effect of ionizing radiation on the healing and remodeling of bone.

Radiation-induced apoptosis is ultimately executed by caspases 3 (Fig. 1) that operate through the mitochondrial-mediated pathway involving cytochrome c release from the mitochondria in order to activate caspase-3 (Eliseev *et al.*, 2005; Hosokawa *et al.*, 2005). The mitochondrial-mediated pathway of apoptosis is regulated by the bcl-2 family (Reed, 2000). The Bcl-2 family consists of both pro-apoptotic members that promote mitochondria permeability such as Bax, Bad and anti-apoptotic members that inhibit function of pro-apoptotic members such as Bcl-2,

Bcl-Xl, Mcl-1, Bcl-W and A1 (Burlacu, 2003). Interactions between pro-apoptotic and pro-survival members of the Bcl-2 family of proteins are decisive in the initiation of mitochondria pore opening (Smith *et al.*, 2008). The members of Bcl-2 family could form either homodimers or heterodimers, suggesting neutralizing competition between these proteins. (Burlacu, 2003) Bax inactivates bcl-2 proteins through heterodimerization. When Bax is excess, cells execute a death command; but, when Bcl-2 dominates, the apoptotic cell death is inhibited and cells survive (Korsmeyer, 1999). Thus, the ratio of bax to bcl-2 proteins increases during the apoptosis induction (Chae *et al.*, 2004; Oltvai *et al.*, 1993; Qiao *et al.*, 1998). In addition, Bad form heterodimers with bcl-xl and bcl-2 during apoptotic process (Burlacu, 2003; Elyaman *et al.*, 2002).

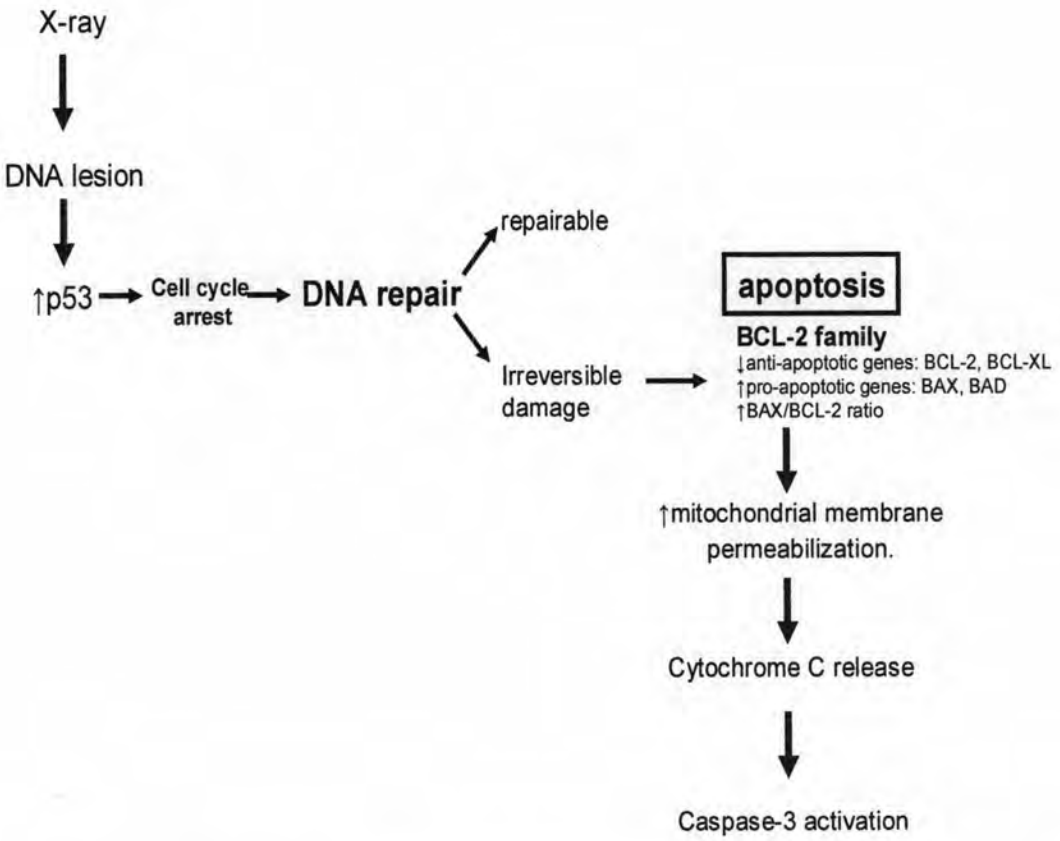

Figure 1 Simplified model of radiation-induced apoptosis pathway. Radiation-induced DNA lesions initiate apoptosis via p53-dependent mechanism, e.g. by regulating the expression of Bcl-2 family members which determine the release of cytochrome c from mitochondria resulting caspase-3 activation.

Most of the studies revealed the effect of high dose radiation (>1Gy) on normal bone cells in a dose dependent manner including the reduction of bone cell proliferation and cell synthetic activity as well as the increase of cell cytotoxicity, several markers of cellular apoptosis and cellular differentiation (Dare *et al.*, 1997; Gal *et al.*, 2000; Margulies *et al.*, 2006). However, there is no scientific data showing the biological effects of dental radiation on primary human bone cells.

The purpose of this study was to determine the effects of short term toxicity of dental radiation on human bone cells as well as the apoptotic-related gene expression.

RESEARCH OBJECTIVES

1. To determine the effect of dental radiation on the cytotoxicity of human bone cells.
2. To examine whether there is any changes of Bcl-2, Bcl-xl, Bax, Bad and Caspase-3 expression in human bone cells irradiated by dental radiation.

RESEARCH HYPOTHESIS.

1. Dental radiation has cytotoxic effect on human bone cells.
2. Dental radiation causes changes in the level of Bcl-2, Bcl-xl, Bax, Bad and Caspase-3 expression in human bone cells irradiated by dental radiation.

FIELD OF RESEARCH

This is an in vitro study performing in cell culture model to determine the effect of dental x-radiation used in dental clinic on primary bone cell cytotoxicity and expression of apoptotic-related gene.

LIMITATION OF RESEARCH

In this study, apoptotic-related gene expression was determined in primary human bone cells. The advantage of primary cell cultures are the closer of their functions and activities to normal cells. However, heterogeneity of the samples obtained from individuals need to be considered. In addition, the results obtained from cell culture study may not be similar to those occur in vivo.

EXPECTED BENEFIT

We anticipate that the results from this study would provide a more precisely understanding of the direct effect of dental radiation on human bone cells and to assess the possibility of detrimental effects of dental radiation on human bone cells in order to develop useful dental radiographic prescribing protocol for patient especially immediately after dental surgery.