CHAPTER I

INTRODUCTION

PRINCIPLES, THEORIES AND RATIONALES

The magnets have been used over the past several years for a variety of purposes in the field of reconstructive medicine (Blechman, 1978). In clinical dentistry, magnetic force systems were initially used to aid retention of dental prosthesis (Behrman, 1960; 1964 and Gilling, 1981; 1983). The magnets used at that time were made of aluminum nickel cobalt or platinum cobalt type, but their uses were limited by their cost, size, and risk of demagnetization.

The introduction of magnets containing rare earth elements, i.e. samarium and neodymium, has led to resurgence of magnet in clinical dentistry, primarily because of high magnetic forces produced by relatively small size and resistance to demagnetization (Robinson 1984). However, they have some shortcomings, such as brittleness, low corrosion resistance, irreversible magnetic loss when heated, and expensiveness (Tsutsui *et al*., 1979).

An increasing number of reports on the use of rare earth magnets as an alternative source of force in orthodontic therapy have been reported, including the movement of individual teeth, the facilitated eruption of impacted teeth, the distal movement of maxillary first and second molars, the application of intermaxillary forces, the orthopedic correction of horizontal, vertical, and transverse jaw discrepancies, and the retention (Muller, 1984; Blechman, 1985; Dellinger, 1986; Kawata *et al*., 1987; Gianelly *et al*., 1989; Sandler *et al*., 1989; Vardimon *et al*., 1989; 1990; Springate and Sandler, 1991; Bondemark and Kurol, 1992; Darendeliler and Joho, 1992; 1993; Darendeliler and Friedli, 1994).

Magnets can eliminate the problem of the patient's cooperation, i.e. wearing elastics, since they are totally operator-controlled. Furthermore, magnets can also be used for continuous high-deflection, low rate force with precise control. They are

friction- free mechanics and have minimum undesirable force vectors. These several advantages draw orthodontists' attention to the use of magnets.

It is well recognized that intraoral rare earth magnets are susceptible to corrosion, and that numerous variables facilitate corrosion (Tsutsui *et al*., 1979; Vardimon and Mueller, 1985; Bondemark *et al*., 1994a; b; Blechman and Steger, 1993; Noar and Evans, 1999). Up until now, the biocompatibility of intraoral magnets and the susceptibility to their corrosion products have still been unclear. Several studies have shown that these magnets have acceptable biocompatibility, while others have demonstrated contradictory results (Camilleri and Mcdonald, 1993; Linder-Aronson *et al*., 1992; 1995; 1996).

Watanakit and Jotikasthira (2001) reported that commercially available magnets could generate the attracting force to orthodontic brackets greater than that generated by orthodontic magnets, and that the compositions of both magnets were comparable. In order to bring these commercial magnets for clinical application, preliminary *in vitro* assessment of the biological effects of magnetic field was conducted by Luewitoonwechkit and Jotikasthira (2003). The results showed that the magnetic field had no short-term effects on the viability and the growth of cultured human gingival fibroblasts and epithelial cells. Meanwhile, the biocompatibility of the corrosion products released from commercial and orthodontic magnets was also conducted by Panichakul and Jotikasthira (2003). They demonstrated that the short-term exposure to corrosion products of both magnets did not affect the viability and the growth of cultured human gingival fibroblasts and epithelial cells, although the commercial magnets showed higher quantities of leachable elements than those released from orthodontic magnets.

It is important to ensure, as long as it is reasonably possible, that any new material destined for clinical use should not produce any adverse effects. Biological safety testing of commercial magnets containing rare earth elements would then need further evaluation of the toxic effects of the leachable corrosion products. Although corrosion products released from commercial magnets have short-term biocompatibility

to cultured human gingival fibroblasts (Panichakul and Jotikasthira, 2003), the cytotoxic effects at the molecular level of cultured human gingival cells are still not thoroughly investigated. In this study, we investigated the cytotoxic effect of corrosion products released from commercial magnets on cultured human gingival epithelial cells. The reason we chose to study the cytotoxic effect on primary human gingival epithelial cells was because these cells directly contact with the corrosion products in the oral environment *in vivo*; thereby, it is appropriate to study the cytotoxic effect on these cells *in vitro*.

The assessment of cell apoptosis *in vitro* is one of the cytotoxicity studies. A large number of qualitative and quantitative assays exist for measuring apoptosis. In this study, we performed both qualitative and quantitative assessments of the cytotoxic effect of corrosion products released from commercial magnets on cultured human gingival epithelial cells in terms of apoptosis as well as necrosis. In order to obtain a more precise picture of the potential cytotoxicity of the corrosion product, the characteristic of nuclear morphology using propidium iodide and fluorescence microscope was studied. This would then allow us to distinguish apoptotic cells from healthy and necrotic cells. The quantitative assessment was performed by a FITC-conjugated annexin V and propidium iodide assay. This relatively simple and accurate technique was developed for detection of apoptosis and necrosis by flow cytometry (Vermes et al., 1995; Lawrence and Jonathan , 2001).

THE OBJECTIVES OF THE STUDY

1. To investigate the morphological alterations of untreated cells and treated cells with corrosion products released from commercial magnets in 0.9% sodium chloride between control and all experimental groups for 5 days.
2. To determine the number of apoptotic and necrotic cells treated with corrosion products released from commercial magnets in 0.9% sodium chloride for 5 days.

3. To compare the number of apoptotic and necrotic cells between two different volumes of corrosion products and control.

THE HYPOTHESES

The null hypotheses, Ho, are:

1. "The number of apoptotic cells treated with corrosion products released from commercial magnets (in 0.9 %NaCl) is not significantly different from that of the control untreated cells".

The hypothesis will be rejected if there are significant differences between the number of apoptotic cells in the presence of corrosion products and the control group (α=0.05).

2. "There is no significant difference between the number of apoptotic cells treated with two different volumes of corrosion products released from commercial magnets"

The hypothesis will be rejected if there are significant differences between the number of apoptotic cells in two different volumes of corrosion products released from commercial magnets (α=0.05).

ANTICIPATED BENEFITS

The findings from this *in vitro* study will be useful to

1. Understand the cellular responses to corrosion products released from magnets. The *in vitro* study of biological effects of magnets on epithelial cells will be useful for future research and clinical application of the magnet in orthodontics.

2. Better understand the cytotoxicity of corrosion products released from available commercial magnets in the complex *in vivo* phenomena.

3. Gain basic knowledge in order to select available commercial magnets and coatings for future use in the field of orthodontics.

SCOPE OF THE STUDY

The biological effect of magnetic materials has been questioned. Consequently, we would like to address the question with 2 specific aims. These include (1) to study the morphological alterations of the epithelial cells among experimental and control groups by propidium iodide (PI) staining under fluorescence microscope, and (2) to study the cytotoxic effect of corrosion products released from commercial magnets on cultured human gingival epithelial cells by flow cytometric analysis.

GLOSSARY OF TERMS

Commercial magnets: The available magnet can attract the orthodontic bracket with strong forces and can be made in various shapes. Previously, it was reported that commercial magnet was composed of iron (Fe), neodymium (Nd), cobalt (Co), copper (Cu), and gadolinium (Gd); moreover, the generated force was greater than the orthodontic magnet.

Corrosion products released from commercial magnets: The corrosion products released from the commercial magnets which were left corroded in 0.9% sodium chloride, the most corrosive solution. It was reported that these corrosion products consisted of various elements, including boron (Br), silicon (Si), iron (Fe), nickel (Ni), cobalt (Co), and copper (Cu) (399.06 ppm, 75.96 ppm, 3.14 ppm, 0.65 ppm, 0.69 ppm, 0.28 ppm, respectively).

Programmed cell death: An active cellular process that culminates in cell death. This may occur in response to development or environment cues, or as a response to physiological damage detected by the cell's internal surveillance networks.

Apoptosis: One type of programmed cell death characterized by a particular pattern of morphologic changes. The name comes from the ancient Greek, referring to shedding of the petals from flowers or leaves from trees. Apoptosis is observed in all metazoans, including both plants and animals, but the genes encoding proteins involved in apoptosis has yet to be detected in single-celled organisms, such as

yeasts. Apoptotic death occurs in two phases. During *the latent phase*, the cell looks morphologically normal but is actively making preparations for death. *The execution phase* is characterized by a series of dramatic structural and biochemical changes that culminate in the fragmentation of the cell into membrane-enclosed *apoptotic bodies*.

Necrosis (Accidental cell death): Cell death that results from irreversible injury to the cell. Cell membranes swell and become permeable. Lytic enzymes destroy the cellular contents, which then leak out into the intercellular space, leading to the augmentation of an inflammatory response.