CHAPTER V
DISCUSSION

The discussion was presented as follows;

Part I: The composition and quantities of corrosion products released from orthodontic magnets and commercial magnets

Part II: Biocompatibility test of corrosion products released from orthodontic magnets and commercial magnets

Part I: The composition and quantities of corrosion products released from orthodontic magnets and commercial magnets

This study demonstrated the composition and quantities of corrosion products released from orthodontic magnets and commercial magnets in three types of medium (DMEM, 0.9% NaCl, and artificial saliva). The quantities of six elements (boron, cobalt, copper, iron, nickel, and silicon) in corrosion products of both magnets were analyzed by an Atomic Absorption Spectrometer. Among three different types of corrosive medium, the orthodontic magnets and commercial magnets were corroded in 0.9% NaCl and artificial saliva more than cell culture medium. There was boron with the highest quantity and silicon was the second highest among six tested elements in corrosion products released from both types of magnets. The iron, nickel, cobalt, and copper appeared with little quantity. Generally, there were corrosion products released from commercial magnets more than orthodontic magnets.

There were corrosion products of the orthodontic magnets and commercial magnets released in 0.9% NaCl and artificial saliva more than cell culture medium. This may because the chemical properties, such as chemical composition and pH, of 0.9% NaCl and artificial saliva can facilitate corrosion process of both magnets. Although there were no obvious differences between corrosion products released in 0.9% NaCl

and artificial saliva, 0.9% NaCl was more slightly corrosive, especially for commercial magnets. The environment containing halide ions, especially chloride ions, contributes to corrosion process (Marek, 1996; Riley *et al.*, 1999). This condition, particularly 0.9% NaCl, does not allow the passivation of magnet's surface, thereby enhancing more corrosion.

The orthodontic magnets are samarium cobalt magnets. The commercial magnets mainly consist of neodymium (Nd) and iron (Fe) (Watanakit and Jotikasthira, 2001). The orthodontic magnets were coated with biocompatible polymer, but commercial magnets were not. There were corrosion products released from commercial magnets more than orthodontic magnets. This was consistent with several reports demonstrating that the uncoated magnets were excessively corroded and the coated samples showed only a small amount of corrosion (Bondemark *et al.*, 1994b; Vardimon and Mueller, 1985). Moreover, the neodymium iron boron magnets do not resist corrosion as well as samarium cobalt magnets do because of high iron content (Blechman and Steger, 1993).

In this study, some elements were chosen to be analyzed by Atomic Absorption Spectrophotometer, i.e. boron, cobalt, copper, iron, nickel, and silicon. This is because these elements were components of both orthodontic magnets and commercial magnets (Wattanakit and Jotikasthira, 2001). Nevertheless, the other compositions for instance samarium, neodymium, and gadolinium, were not included, since the equipment for analyzing these elements was not available. The result of chemical analyses showed that there was boron in the highest quantity and silicon was the second highest. The iron, nickel, cobalt, and copper were trace. Even if there was high quantity of boron (399.06 ppm) released from commercial magnets in this study, this amount of boron was less than the toxic dose (4,161 ppm) recommended by Agency for Toxic Substances and Disease Registry (ATSDR). If large amount of boron (4,161 ppm) is taken over short periods of time, it can affect the stomach, intestines, liver, kidney, and brain and can eventually lead to death. With regard to the boron content, corrosion products of both magnets can be considered to be not critical, but it is doubtful whether the longer exposure time of corrosion products is harmful. Therefore, magnets should be

immersed for long-term periods, such a month or several months, to assess the quantity of corrosion products.

Furthermore, there are many techniques in Atomic Absorption Spectrophotometry divided by atomization process such as flame, graphite furnace, and vapor generation. A proper technique should be selected for each particular experiment according to the qualification of sample. The flame atomization was used in this study because the samples were solution and degradable by temperature. However, this technique has some limitations. These include the concentration of element, the amount of solid particles in solution, the amount of solution, and the chemical and physical properties of solution. In this study, there was little quantity of corrosion extracts remaining for chemical analyses due to some corrosion products had to be used in biological investigation. So, it must be diluted in order to made adequate solution for all of these chemical analyses. This may cause too diluted concentration than the detection limit. Also, the viscosity of solution is another restriction of this technique. The artificial saliva was viscous and may then affect the detection.

Another important aspect which should be considered is the physical property of magnets. The magnet can be destroyed if it is exposed to high temperature close to or exceeding the Curie temperature. Nevertheless, the temperature used for autoclave (121 $^{\circ}$C) is far below the Curie temperature for magnets, that is 680 $^{\circ}$C (Tsutsui, 1979), so the sterile temperature would not affect the magnetic property. In addition, surface polishing should not affect the corrosiveness of these magnets except possibly to decrease the corrosion by providing a smooth and scratch-free surface.

Part II: Biocompatibility test of corrosion products released from orthodontic magnets and commercial magnets

In this *in vitro* study, the trypan blue dye exclusion assay and flow cytometric analysis were methods of choice to evaluate viability and growth of the cultured human gingival fibroblasts, respectively. The viability and growth of the cultured human gingival fibroblasts in a control group and all experiment groups in the presence of corrosion products from both magnets for 3 and 7 days were not significantly different. However,

the growth of human gingival fibroblasts cultured for 7 days was significantly less than 3 days.

The specifications and certification programs up to now have mainly emphasized the physical and chemical properties of the materials, rather than their biological suitability. The demand for biological evaluation of dental materials has been increasing during recent years, because of increasing concern for safety. A few reports about the biological effects of corrosion products released from magnets have been published in the literature and they are somewhat contradictory. From the result of this study in part I, the magnets are more likely to be corroded in oral tissue and the corrosion products may have negative impact on these cells. So, the biocompatibility of these corrosion products should be investigated before clinical application. The initial biological testing should be carried out in *in vitro* systems. The cell culture was performed for this *in vitro* study. The oral tissue mainly consists of epithelium and connective tissue. Fibroblasts are principle cells of connective tissue. While being used in orthodontics, especially in the artificial eruption of unerupted tooth, the magnet contacts adjacent connective tissue. Therefore, the use of human gingival fibroblasts to investigate cytotoxicity in the oral cavity is a more pertinent approach.

The *in vitro* evaluation of biocompatibility of corrosion products released from magnets can be carried out by two different methods with different cell-material contact approaches. These are the millipore filter method and extraction method (Bondemark *et al*, 1994). The extraction method was selected in this study in order to obtain a more precise picture of the potential cytotoxicity of corrosion products released from orthodontic magnets and commercial magnets. The extraction method was to particularly assess corrosion products regardless of possible effects of the static magnetic field on the target cells. There was no magnetic field in the corrosion products. This was also confirmed by measurement of the magnetic flux density of these corrosion products with a teslameter. In addition, it is well known that corrosion of rare earth magnets leads to substance loss and to disturbed physical properties (Tsutsui et al, 1979; Vardimon and Mueller, 1985; Geis-Gerstorfer and Weber, 1987). Moreover, the millipore filter method is based on indirect cell-material contact, where the target

cells grow as a monolayer on one side of the permeable molecular filter and the magnets are placed on the other side of the filter. That magnet has magnetic field which provides a confounding factor for solely measuring the effect of corrosion products. The weight of magnet may also affect cells grown underneath it.

The medium selected for biocompatibility test was the solution containing maximum corrosion products of orthodontic magnets and commercial magnets. Even though the quantities of corrosion products released in 0.9% NaCl and artificial saliva were approximately equal, but corrosion products released in 0.9% NaCl was selected for the biocompatibility test. This is because artificial saliva was viscous and might cause some errors in the detection of chemical analyses as previously described. This high viscosity may also change the proper environment of culture medium. In fact, we observed the inhibitory effect on cell proliferation of the cultured human gingival fibroblasts with addition of artificial saliva regardless of the presence of any corrosion products.

The biocompatibilty test in this study was determined by the effect of corrosion products on the viability and growth of the cultured human gingival fibroblasts. The viability was investigated by staining cells with trypan blue dye. The dye uptake is dependent upon cell membrane integrity and cellular metabolism. Cell viability should be at least 80% as determined by this assay. The result of cell viability in this study showed that the viability of the cultured human gingival fibroblasts in a control group and all experimental groups in the presence of corrosion products released from orthodontic magnets and commercial magnets for 3 and 7 days was not significantly different. In addition, the viability of each group was more than 80%. So the exposure of corrosion products for 3 and 7 days did not have any deleterious effect on the viability of the cultured human gingival fibroblasts. Nevertheless, the trypan blue dye exclusion method is a rough estimation of cell viability, because cells that exclude dye are not necessarily capable of attachment and prolonged survival or proliferation (Spector, 1988).

The cell growth was measured by the percentage of new DNA synthesis (S phase) with immunofluorescent staining of incorporated BrdU and flow cytometry. When fibroblasts were stimulated with Phorbol12-myristate13-acetate, a Tumor Promoting

Agent (TPA), this agent could significantly induce proliferation of the cultured human gingival fibroblasts. This indicates that the flow cytometer has capability to detect the changes in new DNA synthesis. Therefore, TPA can be used as a positive control for the flow cytometry. The results of this study revealed that the growth of the cultured human gingival fibroblasts in a control group and all experimental groups in the presence of corrosion products released from orthodontic magnets and commercial magnets was not significantly different. Therefore, corrosion products of both types of magnets had no effect on the growth of the cultured human gingival fibroblasts.

When comparing between experiment time intervals, the growth of the cultured human gingival fibroblasts at 3rd day was greater than that at 7th day. There are three main reasons that may explain this finding. First, the longer culture time the cell spends the more metabolic products the cell generates. The environment of culture medium becomes more unfavorable for cell proliferation. The cells continue to grow until they enter the "early stationary phase" in which the cell's division rate decreases. This may be due to nutrient depletion or accumulation of deleterious waste products (Jakoby and Pastan, 1979). Second, cell proliferation must be regulated, so it can maintain both the number of cells and their spatial organization. There are two types of signals that control the cell division, i.e. the local cell population density and the cell-to-cell attachment. These can be demonstrated in the simplified condition of fibroblast cell culture. The cell divides until a confluent monolayer is formed in which each cell is attached to the dish and contacts its neighbors. At this stage, normal cells stop dividing. This phenomenon is known as density-dependent inhibition or contact inhibition of cell division (Andrew, 1994). A growth curve of mammalian cells in suspension culture is a semilogarithmic sigmoidal plot of cell density (cells per ml of culture medium) as a function of time. Third, normal *in vitro* cells have a finite lifespan. The process of cellular aging is considered to be a deterioration of the cellular processes that are necessary to support continuing replication (Jakoby and Pastan, 1979). However, the results from this study showed that the corrosion products released from orthodontic magnets and commercial magnets did not significantly affect the viability and growth of the cultured human gingival fibroblasts. This means that corrosion products of magnets may have good

biocompatibility and this is in agreement with previous *in vitro* studies. Similarly, neodymium-iron-boron magnets do not appear to have cytotoxic effects on osteoblast-like cells (UMR-106)(Sandler *et al.*, 1989). Another report showed less proliferation in the presence of $Nd_2Fe_{14}B$ magnetic corrosion products; however, cellular attachment was not disrupted after an experimental period of 48, 96, and 144 hours (Evans and McDonald, 1995).

Several previous studies have shown detoxification of toxic metals by metallothioneins (MTs) (Lau *et al.*, 2001; Coyle *et al.*, 2002). MTs are ubiquitous, low molecular weight, cysteine-rich, metal-binding proteins that are induced by many agents including metals. Any increase in MTs might be interpreted as a protective mechanism in which high local concentrations of metals bind to them and so are prevented from causing local or systemic toxic effects. The synthesis of MTs in cells adjacent to the particles of metal would be expected to regulate initially after exposure to toxic metal. This mechanism may in part play a role in detoxification of toxic metals in corrosion products. The studies involving with the association between MTs expression and corrosion products should be further investigated.

Although the corrosion products of orthodontic magnets and commercial magnets were not cytotoxic to the cultured human gingival fibroblasts, the magnets should preferably be coated with any substance. For clinical application, the magnets may be in the oral cavity for a long time and they are likely corroded. Thus, the coating of magnets with biocompatible epoxy resin (Blechman, 1985), stainless steel (Cerny, 1978) or a thin layer of parylene (Vardimon *et al.*, 1991) is recommended in order to decrease the oral exposure of corrosion products.

LIMITATION OF THIS INVESTIGATION

1. The light source is one of important components of the Atomic Absorption Spectrophotometry. This must be specific for each tested element. The light source of some elements is expensive such as neodymium, samarium, etc. So, this study could only determine the quantities of six elements in corrosion products.

2. The size of commercial magnets was rather small because they were prepared according to the surface area of orthodontic magnets. The amount of medium used for immersion was limited. It was not sufficient and must have been diluted before being analyzed by a whole series of chemical analyses. Therefore, the final concentration might be less than the detection limit of measurement.

3. The *in vitro* study with cell culture requires technical experience. There are numerous factors influencing cell culture procedures. The control of these variables is essential for experimental condition.

4. The duration of exposure time to corrosion products was restricted because the cells only took a week to become confluent.

5. The cost of materials, such as chemical reagents, is expensive, so few experiments could be repeated in this study.

SUGGESTIONS FOR FURTHER STUDY

1. There were corrosion products released from uncoated commercial magnets more than those from coated orthodontic magnets. The future study may determine the composition and quantities of corrosion products released from commercial magnets coated with various substances.

2. The corrosion products of magnets should be analyzed by the more precise equipment that can detect all of elements in the corrosion products.

3. In the oral cavity, magnets may contact with other cell types such as oral epithelial cells. Corrosion products may then affect a number of different cell types. The *in vitro* biological evaluation about corrosion products of magnets also should be conducted on other cells as well.

4. Orthodontic treatment with magnets may continue for several months and there are very few reports on the exposure time. It would be of particular interest to investigate possible biological effects of long-term exposure to corrosion products released from magnets on oral tissues and cells.

5. The effect of various amount of corrosion products on the cell viability and cell growth should be studied in order to evaluate a dose response effect.

6. Although the orthodontic magnets and commercial magnets did not affect the viability and growth of the cultured human gingival fibroblasts, the cytotoxicity of magnets should be evaluated by determining the apoptosis of cells. This will be useful to certainly assure the biological safety of magnets.

7. Another further level of biological testing, i.e. *in vivo* and/or clinical trial, should be conducted to better understand the biocompatibilty of corrosion products released from magnets.