CHAPTER V
DISCUSSION

Discussion on results of the study

The results of this *in vitro* study indicated that the viability and the growth of the cultured human gingival fibroblasts were not significantly influenced by the static magnetic field.

I. The viability of the cultured human gingival fibroblasts under static magnetic field

It was shown that there was no statistically significant difference between the viability of the cultured human gingival fibroblasts grown under the static magnetic field and without magnetic field (P=0.58) at both day 3 (P=0.33) and day 7 (P=0.53)(Table 4.1 - 4.4).

Nevertheless, there were some noticeable differences in the median values of the viability at day 3. The median value of the viability of Group 4 was only 87.00 %, while those of the other four groups were higher than 95.80 %. In principle of dye exclusion method, this difference could be ignored because 10 – 20 % of difference was still regarded to be within the limit of insignificant difference (Darlington, 1988). Moreover, the correlation coefficients (r) (Table 4.5) and the scatter diagrams (Figure 4.1) were also showed that there was no significant correlation between the viability of the cultured human gingival fibroblasts and the extent of magnetic field intensity, at both low level (r = 0.15, P=0.54) and high level (r = -0.21,P=0.41) of magnetic fields. Therefore, it could be assured that static magnetic field had no significant effects on the viability of the cultured human gingival fibroblasts.

II. The growth of the cultured human gingival fibroblasts under static magnetic field

The statistical comparisons of the growth of the cultured human gingival fibroblasts among five experimental groups showed that the growth of the cultured human gingival fibroblasts under magnetic field was not significantly different from those

without magnetic field (P=0.98) at both day 3 (P = 0.96) and day 7 (P = 0.96) (Table 4.6 - 4.9).

At 7 days of exposure time to magnetic field; however, it was found that there were some remarkable differences between the median values of the growth of the cultured human gingival fibroblasts of Group 2 (Median = 5.56), as well as Group 5 (Median = 8.10), and those of other groups (Medians of Group 1, 3, and 4 = 10.57, 10.28, and 11.15 respectively) (Table 4.8). Those differences might be caused by an error of statistical analysis. There was an inequality of the number of repeated experiments in each experimental group, Group 3 and Group 5 was each repeated for only three times, while the other three groups was four to five times, and it could be noticed that the fewer repeated experiments were coincident with the decreased median values (Table 4.8). Moreover, the quartiles (P_{25}, P_{75}) of the five experimental groups were still not much different compared to the differences of median values. Therefore, it can be concluded that the growth of the cultured human gingival fibroblasts grown under static magnetic field for 7 days was not different from that without magnetic field.

From the results of present study, it was also found that there was a statistically significant difference between the growth of the cultured human gingival fibroblasts at day 3 and day 7 ($P < 0.00$). This difference of growth also occurred in the control group, the cultured human gingival fibroblasts grown without magnetic field. It indicated that the decreased multiplication in the cultured cells was not caused by the magnetic field effect. The difference of growth might be affected by some factors, i.e. the density-dependent inhibitory effect, the cellular transformation, or some environmental factors, which often occur in cell population with extended culture time (Jakoby and Pastan, 1979).

Moreover, the correlation coefficients (r) (Table 4.9) and the scatter diagrams (Figure 4.2) were also showed that there was no significant correlation between the growth of the cultured human gingival fibroblasts and the extent of magnetic field intensity, at both low (r = 0.05, P=0.85) and high level (r = 0.01, P=0.98) of magnetic

fields. Therefore, it could be concluded that static magnetic field had no significant effects to the growth of the cultured human gingival fibroblasts.

Therefore, from overall results of present study, it could be primarily assumed that the static magnetic field had an acceptable biocompatibility. This finding was found to be in accordance with several studies, which indicated that magnetic fields were safe to be used clinically (Tsutsui *et al.*, 1979; Sandler *et al.*, 1989; Blechman and Smiley, 1978; Camilleri and McDonald, 1993; and Bondemark *et al.*, 1994b, 1994c, 1995a, 1995b, 1998).

Nevertheless, some biological changes influenced by exposure of static magnetic field were reported. It was found that the magnetic field might cause thinner of epithelium, more bone resorption (Linder-Aronson and Lindskog, 1991; Linder-Aronson *et al.*, 1992,1995), greater root resorption, increased width of the PDL, more bone remodeling (Tengku *et al.*, 2000) and higher rate of tooth movement. Moreover, the reduction in serum calcium and elevation of the white blood cell count (Darendeliler *et al.*, 1995), were found as well.

There was an *in vitro* study, whose experimental design was similar to the present study, indicated that magnetic fields had some effects on the cellular growth and cellular attachment of the human periodontal fibroblasts (Linder-Aronson and Lindskog, 1995). However, the dimension of magnets, the generated magnetic fields, the exposure time and the cell types used in the experiments were different. The experiments of Linder-Aronson and Lindskog were made in longer duration of 5 weeks, in addition, the magnets employed in their study were much greater in size (32 mm in diameter with 7 mm in thickness), and could generate 160 to 280 mT of magnetic field, while the magnets in dimension of 7 mm in diameter with 2 mm in thickness used in this study generated only 62.0 to 98.6 mT. Moreover, it was found that although the gingival fibroblasts and the periodontal fibroblasts appeared to be similar in their morphology, some differences such as sizes and granularity of cultured cells (Kuru *et al.*, 1998), proliferation rates of passaged cultures, and the biosynthetic activity including productions of protein and collagen were indicated (Somerman *et al.*, 1988).

Therefore, it can be concluded that the viability and the growth of the cultured human gingival fibroblasts were not affected by static magnetic field, generated by magnets, whose dimensions are applicable for orthodontic treatment in short term.

Limitations of the research

1) Pattern of magnets placement

The static magnetic fields used in this study were generated by two patterns of magnets placement, the single and the attractive magnets. Another pattern of magnets placement, the repulsive or repelling magnets, was not selected because of the limitations in placement of magnets during the process of magnetic field measurement and the cell culture experiments. Furthermore, it was indicated that the flux density between two magnets in repulsive action was much less than both the single magnet and attractive magnets. The magnitude of flux density at the midway between the pair of repelling magnets was very low or none (Bondemark *et al.*, 1995b).

2) Culture time

The technique of tissue culture allows a more easily controlled test situation and higher statistical accuracy than when an in vivo system used. Consequently, the *in vitro* study had become the technique of choice in present research. However, the culture duration in an *in vitro* study was limited, because the more it was extended the more it could be disturbed by uncontrollable and unknown factors (Jakoby and Pastan, 1979). Therefore, the study was performed in short term for the most reliable results.

Suggestion for further studies

Full evaluation of a dental material should be included of three levels of testing. The Level I is *in vitro* testing considered as a screening phase in order to detect the toxic, allergic or carcinogenic nature of the material. Level II is an "in-use" test or tests in animals at sites adjacent or surrounding the material. Level III is clinical trials in humans after having sufficient toxicity data from the Level I and Level II (Autian, 1974)

Biological safety testing of static magnetic field on cultured human gingival fibroblasts in this research was regarded as a Level 1 testing and it was done in short

term. Therefore, it should be performed with other kind of cells or observed in a longer of exposure time and higher level of testing to reassure whether the magnets was appropriated to be used clinically.