CHAPTER IV

RESULTS

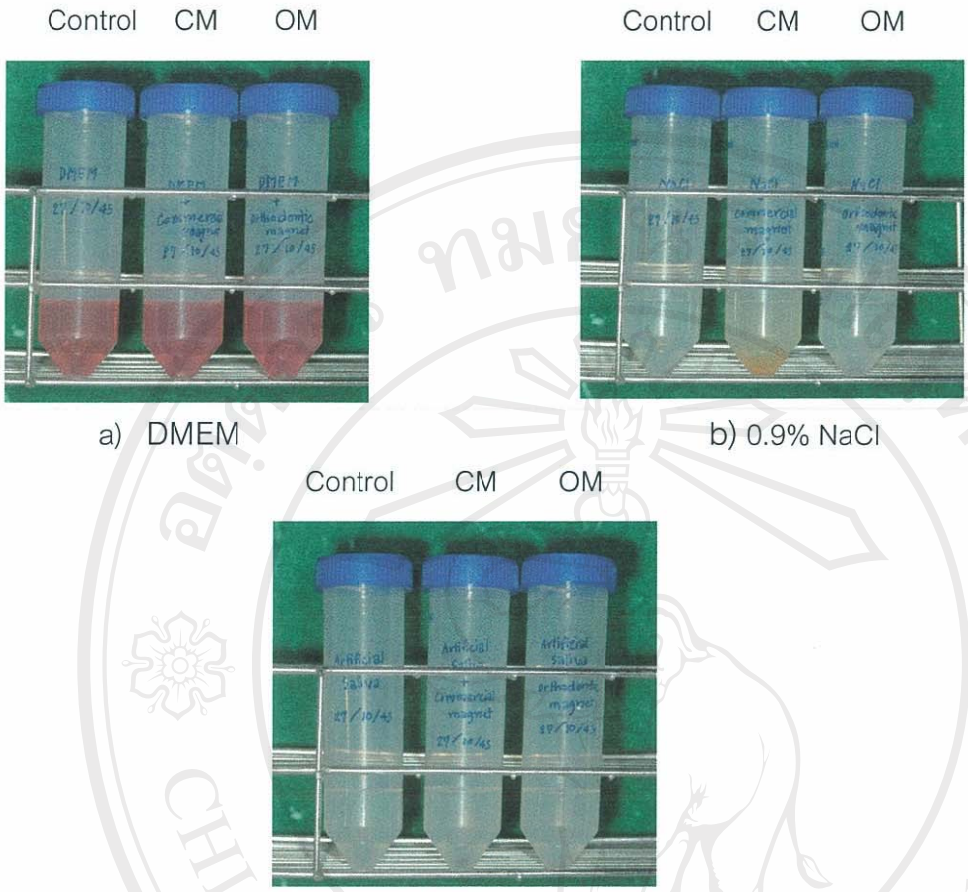
The results of this study were sequentially presented as follows:

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.

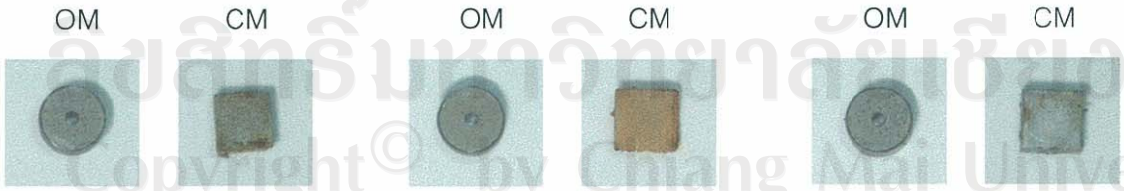
Part I: The composition and quantities of corrosion products released from orthodontic magnets and commercial magnets

There were both water soluble and insoluble corrosion products in three types of solution, i.e. DMEM, 0.9% NaCl, and artificial saliva after orthodontic magnets and commercial magnets were immersed for 7 days. The commercial magnet immersed in 0.9%NaCl was most extensively corroded, and a lot of brownish deposits were formed in the medium (Figure 4.1). The post-immersion specimens of both magnets in each medium were shown in Figure 4.2. The appearance between orthodontic magnets immersed in different types of solution was less marked difference; however, the surface appearance of commercial magnets showed that corrosion had obviously occurred, especially in 0.9% NaCl.



CM = commercial magnet, OM = orthodontic magnet

Figure 4.1 The orthodontic magnets and commercial magnets in each corrosive medium after immersed for 7 days



CM = commercial magnet, OM = orthodontic magnet

Figure 4.2 The specimens of orthodontic magnets and commercial magnets after immersed for 7 days

Table 4.1 Means and standard deviations of the quantities (ppm) of various elements in the composition of the corrosion products released from orthodontic magnets and commercial magnets immersed in three types of medium

Elements	Mean (SD) of quantities (ppm) of elements											
	DMEM				0.9% NaCl				Artificial Saliva			
	Orthodontic magnet		Commercial magnet		Orthodontic magnet		Commercial magnet		Orthodontic magnet		Commercial magnet	
Boron (B)	108.38	(33.34)	136.03	(45.90)	130.24	(39.89)	399.06	(91.74)	199.71	(46.86)	300.11	(7.56)
Silicon (Si)	45.30	(36.44)	65.85	(50.55)	67.59	(10.58)	75.96	(12.91)	78.46	(75.19)	101.66	(91.09)
Iron (Fe)	0.48	(0.11)	1.45	(0.11)	0.88	(0.32)	3.14	(1.63)	0.47	(0.62)	0.36	(0.10)
Nickel (Ni)	0.24	(0.29)	0.49	(0.30)	0.63	(0.23)	0.65	(0.37)	0.77	(0.09)	0.71	(0.23)
Cobalt (Co)	0.50	(0.17)	0.34	(0.09)	0.49	(0.28)	0.69	(0.28)	0.24	(0.11)	0.47	(0.33)
Copper (Cu)	0.19	(0.07)	0.18	(0.14)	0.12	(0.09)	0.28	(0.18)	0.15	(0.15)	0.08	(0.08)

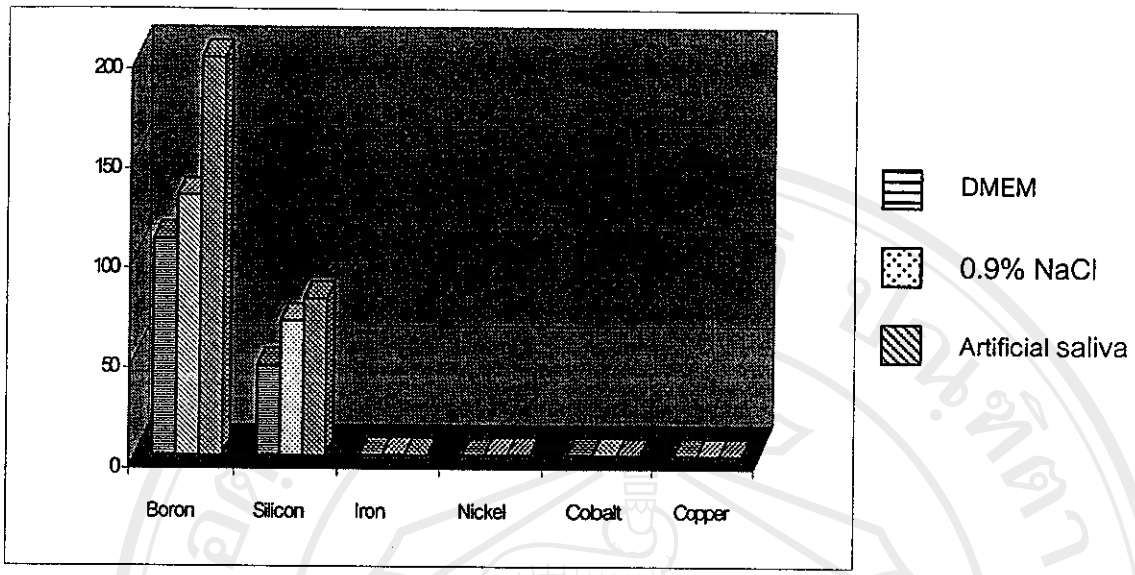


Figure 4.3 The quantities (ppm) of various elements (B, Si, Fe, Ni, Co, and Cu) in corrosion products released from orthodontic magnets

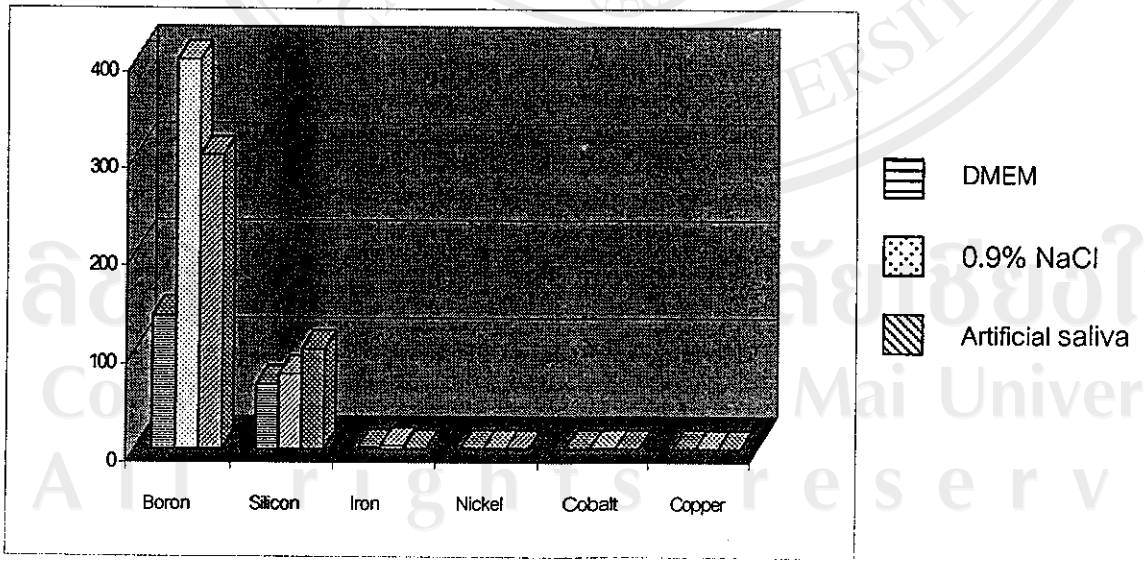


Figure 4.4 The quantities (ppm) of various elements (B, Si, Fe, Ni, Co, and Cu) in corrosion products released from commercial magnets

The quantities of the six tested elements (B, Si, Fe, Ni, Co, and Cu) in corrosion products released from orthodontic magnets and commercial magnets in three types of corrosive medium (DMEM, 0.9% NaCl, and artificial saliva) measured by Atomic Absorption Spectrophotometer were summarized in Table 4.1. The orthodontic magnets and commercial magnets were corroded in 0.9% NaCl and artificial saliva more than cell culture medium. The quantities of tested elements in corrosion products released from both types of magnets had no obvious differences between 0.9% NaCl and artificial saliva except boron and silicon. The quantity of boron released from commercial magnet was 399.06 and 300.11 ppm in 0.9% NaCl and artificial saliva, respectively. However, the 0.9% NaCl was more slightly corrosive, especially for commercial magnets.

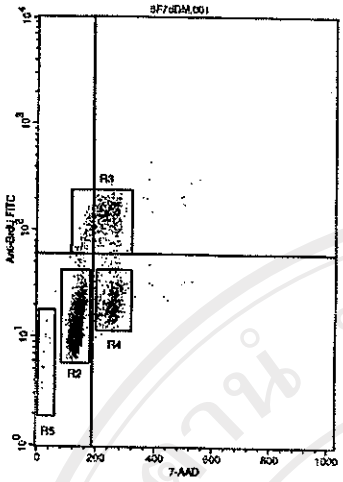
The corrosion products in 0.9% NaCl consisted of boron (130.24 and 399.06 ppm), silicon (67.59 and 75.96 ppm), iron (0.88 and 3.14 ppm), nickel (0.63 and 0.65 ppm), cobalt (0.49 and 0.69 ppm), and copper (0.12 and 0.28 ppm) (for orthodontic magnet and commercial magnet, respectively). Boron was the highest quantity among the tested elements released from both types of magnet, and silicon was the next highest. The concentrations of released Fe, Ni, Co, and Cu ions were trace (Figure 4.3 and 4.4). The corrosion products released from the commercial magnets were generally greater than those released from the orthodontic magnets.

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

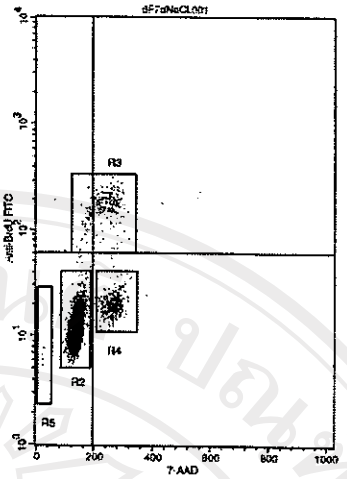
This study investigated the biological effect of corrosion products released from orthodontic magnets and commercial magnets on the cultured human gingival fibroblasts. Six groups were conducted according to the types of magnet and corrosive medium for 3- and 7-day interval of exposure. The percentage of viable cells was measured by trypan blue dye exclusion assay. The rate of new DNA synthesis (S phase) was evaluated by flow cytometric analysis. The experiments were repeated at least three times for each group. The 7-AAD versus BrdU dot plot diagram demonstrated the total DNA in X-axis and the incorporated BrdU levels in Y-axis with gated regions. Each region related to phase of cell cycle (R2= G_0/G_1, R3 = S, R4 = G_2/M, and R5 = Apoptosis). The samples of 7-AAD versus BrdU dot plot diagram were shown that S phase (R3) of a control group and all experiment groups was not obviously different (Figure 4.5.1-4.5.6). The raw data of the viability and growth of the cultured human gingival fibroblasts in each experiment were presented in Appendix. The medians and quartiles (P_{25}, P_{75}) of these viability and growth were shown in Table 4.2.

The medians of the percentage of viability showed that the viability of the cultured human gingival fibroblasts in the control group and all experiment groups in the presence of corrosion products released from orthodontic magnets and commercial magnets for 3 and 7 days was slightly different and in the range of 90-100%.

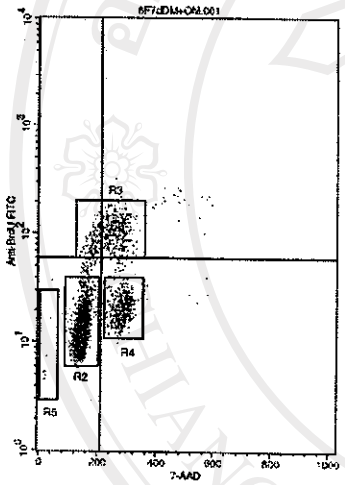
The medians of the percentage of S phase (new DNA synthesis) showed the growth of the cultured human gingival fibroblasts in the control group and all experiment groups in the presence of corrosion products released from orthodontic magnets and commercial magnets for 3 and 7days was slightly different and in the range of 20-27% (for 3 days) and 7-15% (for 7 days).



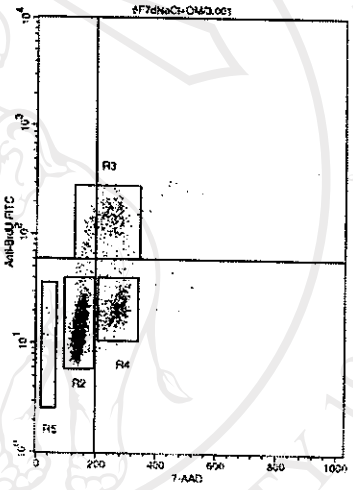
4.5.1) DMEM (Control)



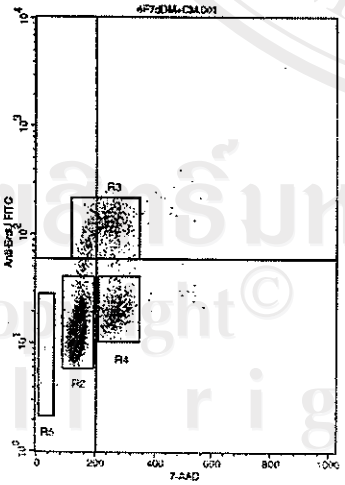
4.5.4) 0.9% NaCl



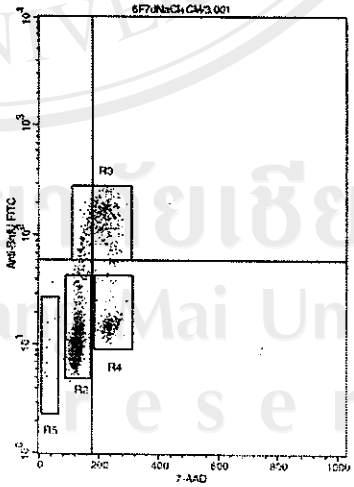
4.5.2) DMEM + Orthodontic magnet



4.5.5) 0.9% NaCl + Orthodontic magnet



4.5.3) DMEM + Commercial magnet



4.5.6) 0.9% NaCl + Commercial magnet

Figure 4.5 The samples of 7-AAD versus BrdU dot plot diagram of a control group and all experimental groups

Table 4.2 Medians and quartiles (P_{25}, P_{75}) of the percentages of the viability and growth of the cultured human gingival fibroblasts in the presence of corrosion products for 3 and 7 days by groups

Cultured Times	Groups	Viability (%)			Growth (%)		
		Median	P_{25}	P_{75}	Median	P_{25}	P_{75}
3 days	DMEM (Control)	94.81	94.28	98.72	20.56	19.52	34.16
	DMEM + OM	100.00	92.50	100.00	20.82	17.86	36.60
	DMEM + CM	93.28	87.73	98.91	26.10	16.67	40.97
	0.9% NaCl	97.92	78.51	100.00	23.82	18.38	33.70
	0.9% NaCl + OM	96.43	88.44	100.00	20.49	15.07	35.91
	0.9% NaCl + CM	97.37	90.65	100.00	20.99	20.94	44.37
7 days	DMEM (Control)	96.28	90.59	100.00	12.65	7.76	19.02
	DMEM + OM	95.00	89.75	97.23	8.42	8.13	17.59
	DMEM + CM	96.28	92.99	97.92	13.21	7.92	17.93
	0.9% NaCl	94.15	88.10	98.64	9.91	3.84	15.49
	0.9% NaCl + OM	100.00	97.76	100.00	13.41	5.15	21.21
	0.9% NaCl + CM	96.31	89.92	100.00	14.69	9.07	21.11

OM = Orthodontic magnet

CM = Commercial magnet

Table 4.3 The comparison of mean ranks of the percentages of the viability of the cultured human gingival fibroblasts by groups

Groups	N	Median	P_{25}	P_{75}	Mean rank	P-value*
DMEM (Control)	10	94.81	94.12	100.00	28.50	
DMEM + OM	9	96.30	91.00	100.00	30.00	
DMEM + CM	10	95.83	89.85	97.92	26.50	0.64
0.9% NaCl	10	95.29	88.10	100.00	26.30	
0.9% NaCl + OM	10	100.00	95.99	100.00	38.20	
0.9% NaCl + CM	10	96.31	90.18	100.00	30.50	

* Kruskal Wallis test

OM = Orthodontic magnet, CM = Commercial magnet

n = Number of repeated experiments

When comparing the viability between all groups without considering the experiment time intervals (Table 4.3), the Kruskal Wallis test indicated 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 was not significantly different (P = 0.64).

Table 4.4 The comparison of mean ranks of the percentages of the viability of the cultured human gingival fibroblasts by cultured times (the experimental time intervals)

Cultured times	n	Median	P_{25}	P_{75}	Mean rank	P-value*
3 days	24	95.75	91.40	100.00	30.73	0.78
7 days	35	96.55	93.55	100.00	29.50	

* Mann-Whitney U test

OM = Orthodontic magnet, CM = Commercial magnet

n = Number of repeated experiments

When comparing the viability regardless of the presence of any corrosion products between 3- and 7-day experiments (Table 4.4), the Mann-Whitney U test indicated that the viability of the cultured human gingival fibroblasts incubated for 3 and 7 days was not significantly different (P = 0.78).

Table 4.5 The comparison of mean ranks of the percentages of the viability of the cultured human gingival fibroblasts in the presence of corrosion products for 3 and 7 days by groups

Cultured Times	Groups	n	Median	P_{25}	P_{75}	Mean rank	P-value*
3 days	DMEM (Control)	4	94.81	94.28	98.72	11.88	
	DMEM + OM	4	100.00	92.50	100.00	15.50	
	DMEM + CM	4	93.28	87.73	98.91	9.75	0.91
	0.9% NaCl	4	97.92	78.51	100.00	13.00	
	0.9% NaCl + OM	4	96.43	88.44	100.00	12.00	
	0.9% NaCl + CM	4	97.37	90.65	100.00	12.88	
7 days	DMEM (Control)	6	96.28	90.59	100.00	18.00	
	DMEM + OM	5	95.00	89.75	97.23	13.20	
	DMEM + CM	6	96.28	92.99	97.92	16.92	0.22
	0.9% NaCl	6	94.15	88.10	98.64	14.08	
	0.9% NaCl + OM	6	100.00	97.76	100.00	27.17	
	0.9% NaCl + CM	6	96.31	89.92	100.00	17.83	

* Kruskal Wallis Test

OM = Orthodontic magnet, CM = Commercial magnet

n = Number of repeated experiments

The comparisons between groups in each experiment time interval (3 and 7 days) were shown in Table 4.5. The Kruskal Wallis test indicated 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 (P= 0.91 and 0.22, respectively).

Table 4.6 The comparison of mean ranks of the percentages of the growth (the rate of new DNA synthesis) of the cultured human gingival fibroblasts by groups

Groups	n	Median	P_{25}	P_{75}	Mean rank	P-value*
DMEM (Control)	9	19.32	10.59	22.13	23.67	
DMEM + OM	6	17.73	8.35	24.77	22.17	
DMEM + CM	7	16.67	10.14	26.10	23.71	0.99
0.9% NaCl	9	16.84	6.97	23.82	21.44	
0.9% NaCl + OM	8	20.10	8.10	21.41	23.75	
0.9% NaCl + CM	7	20.94	13.77	22.94	26.57	

* Kruskal Wallis test

OM = Orthodontic magnet, CM = Commercial magnet

n = Number of repeated experiments

When comparing the rate of new DNA synthesis (S phase) between all groups without considering the experiment time intervals (Table 4.6), the Kruskal Wallis test indicated 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 (P = 0.99).

Table 4.7 The comparison of mean ranks of the percentages of the growth (the rate of new DNA synthesis) of the cultured human gingival fibroblasts by cultured times (the experimental time intervals)

Cultured times	n	Median	P_{25}	P_{75}	Mean rank	P-value*
3 days	21	21.00	19.50	36.66	33.10	< 0.001***
7 days	25	11.07	7.34	18.04	15.44	

* Mann-Whitney U test

n = Number of repeated experiments

When comparing the rate of new DNA synthesis (S phase) regardless of the presence of any corrosion products between 3- and 7-day experiments (Table 4.7), the Mann-Whitney U test indicated that the growth of the cultured human gingival fibroblasts incubated for 3 and 7 days was significantly different ($P < 0.001$).

Table 4.8 The comparison of mean ranks of the percentages of the growth (the rate of new DNA synthesis) of the cultured human gingival fibroblasts in the presence of corrosion products for 3 and 7 days by groups

Cultured times	Groups	N	Median	P_{25}	P_{75}	Mean rank	P-value*
3 days	DMEM (Control)	4	20.56	19.52	34.16	10.25	
	DMEM + OM	3	20.82	17.86	36.60	9.33	
	DMEM + CM	3	26.10	16.67	40.97	12.33	0.95
	0.9% NaCl	4	23.82	18.38	33.70	11.75	
	0.9% NaCl + OM	4	20.49	15.07	35.91	9.50	
	0.9% NaCl + CM	3	20.99	20.94	44.37	13.33	
7 days	DMEM (Control)	5	12.65	7.76	19.02	14.00	
	DMEM + OM	3	8.42	8.13	17.59	12.00	
	DMEM + CM	4	13.21	7.92	17.93	14.00	0.87
	0.9% NaCl	5	9.91	3.84	15.49	9.60	
	0.9% NaCl + OM	4	13.41	5.15	21.21	13.00	
	0.9% NaCl + CM	4	14.69	9.07	21.11	15.75	

* Kruskal Wallis Test

OM = Orthodontic magnet, CM = Commercial magnet

n = Number of repeated experiments

The comparisons between groups in each experiment time interval (3 and 7 days) were shown in Table 4.8. The Kruskal Wallis test indicated 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 for 3 and 7 days was not significantly different (P= 0.95 and 0.87, respectively).

In conclusion, 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 that for 3 days. Consequently, the corrosion products of orthodontic magnets and commercial magnets had no significant effect on the viability and growth of the cultured human gingival fibroblasts.