CHAPTER IV

RESULTS

MTT assay

Human dental pulp cells were cultured in the presence of various concentrations of fluocinolone acetonide (0.1, 1, 10, 50 μM) for 24, 48 and 72 hours. Cell proliferation was determined by MTT assay. Data was shown as mean ± SD of number of viable cells from at least three triplicated experiments. The data in the experimental and control groups of all concentrations were presented in table 1.

Concentrations of fluocinolone acetonide (μM)	Number of viable cells (Mean ± S.D.) $x10^4$		
	24 hours	48 hours	72 hours
50	3.327 ± 0.177	3.484 ± 0.204	3.519 ± 0.011
10	3.667 ± 0.126	3.637 ± 0.151	4.093 ± 0.137
1	3.738 ± 0.184	3.834 ± 0.126	4.277 ± 0.116
0.1	3.647 ± 0.179	4.070 ± 0.172	4.580 ± 0.088
Control (Serum free medium)	3.526 ± 0.056	3.555 ± 0.043	3.780 ± 0.185

Table 1: The effect of fluocinolone acetonide on human dental pulp cell proliferation examined by MTT assay at 24, 48 and 72 hours

At all time periods, the data indicated that human dental pulp cell proliferation in the experimental groups was higher than in control and was shown as reverse dose–

dependent manner, except in a 50 μM group. The experiment was done as a pilot study to select the suitable concentrations of fluocinolone acetonide used for further experiments. Therefore, the concentrations between 0.1 to 10 μM of fluocinolone acetonide were chosen.

Type I collagen synthesis

The ability of human dental pulp cells to synthesize and secrete type I collagen was examined by Western blot analysis. The synthesis of type I collagen in human dental pulp cells was influenced by fluocinolone acetonide (Fig. 2A). The mean amount of type I collagen secreted by human dental pulp cells after being treated with selected concentrations of fluocinolone acetonide for 5 days was shown in figure 2B. The results clearly demonstrated that 1 and 10 μM of fluocinolone acetonide could significantly increase the synthesis of type I collagen approximately 2-fold when compared with the control which was considered as 100% ($p < 0.05$).

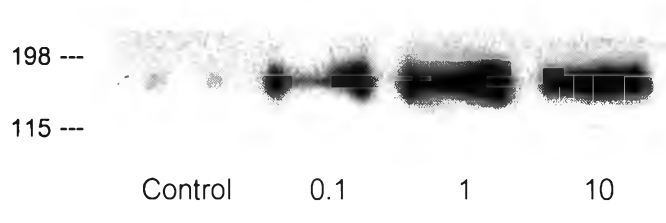

Figure 1: Type I collagen synthesis from human dental pulp cells cultured with 0.1, 1 and 10 μM fluocinolone acetonide at 5 days, examined by Western blot analysis

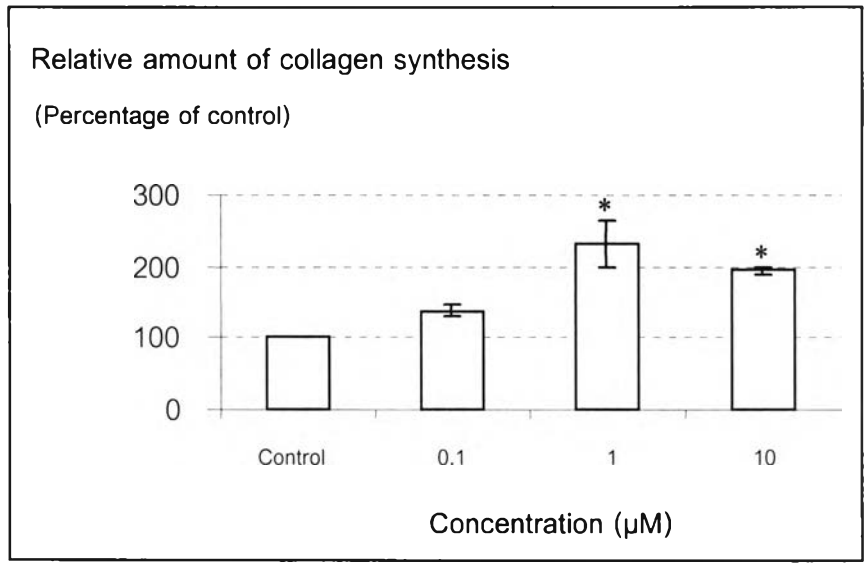

Figure 2: The effect of 0.1, 1 and 10 μM fluocinolone acetonide on type I collagen synthesis detected by Western analysis. Human dental pulp cells were treated with fluocinolone acetonide for 5 days. Fluocinolone acetonide at 1 and 10 μM increased the amount of type I collagen in while 0.1 μM had no significant effect. The relative amount of type I collagen from three separate experiments was shown in mean percentage ± standard deviation. (*) = statistically significant difference from the control group at $p < 0.05$ (Scheffe's test).

The effect of fluocinolone acetonide on the type I collagen synthesis was then confirmed by investigating the expression of type I collagen mRNAs in human dental pulp cells. At 48 hours, 1 μM fluocinolone acetonide could stimulate type I collagen mRNA expression. The expression of GAPDH was used as internal control (Fig. 3). The

activity of type I collagen mRNA expression was significantly increased about 2.8-fold compared with the control ($p < 0.05$) as shown in figure 4.

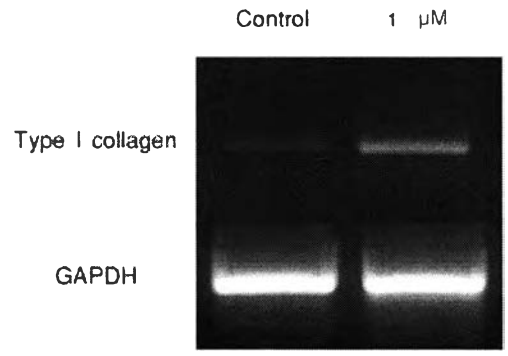

Figure 3: The expression of type I collagen of cultured human dental pulp cells at 48 hours cultured with 1 μM fluocinolone acetonide, *Upper row*: Type I collagen mRNA expression of cultured human dental pulp cells at 48 hours, cultured with 1 μM fluocinolone acetonide; *Lower row*: GAPDH mRNA expression was used as internal control.

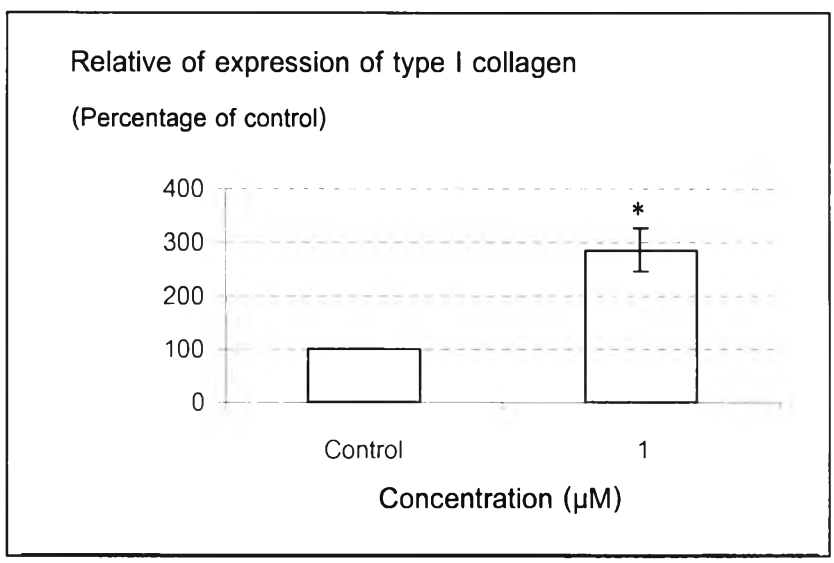

Figure 4: The effect of 1 μM fluocinolone acetonide on type I collagen mRNA expression detected by RT-PCR. Human dental pulp cells were treated with fluocinolone acetonide for 48 hours. Fluocinolone acetonide at 1 μM increased the expression of type I collagen mRNAs when compared to control. The relative amount of type I collagen mRNA expression from three separate experiments was shown in mean percentage ± standard deviation. (*) = statistically significant difference from the control group at $p < 0.05$ (t test).

In vitro calcification

Human dental pulp cells were cultured with selected concentrations (0.1, 1 and 10 μM) of fluocinolone acetonide for up to 28 days. Under phase contrast microscope, morphology of the cells and calcified nodules formation were observed. Initially, most of dental pulp cells in primary culture were fibroblast-like with a few short processes. Some of them gradually became broad and flattened. The cells became confluent at about one week of the cultures (Fig. 5A). When maintained in cultures, the pulp cells slowly became multilayered (Fig. 5B) and clustered forming many nodules (Fig. 5C). Calcified nodules were observed at about 2 weeks (Fig. 5D). The size and number of mineralized nodules were slowly increased with time. At the end of culture, alizarin red S positive staining was observed in all groups, and calcified nodules in the experimental groups seem not different from the controls (Fig. 6).

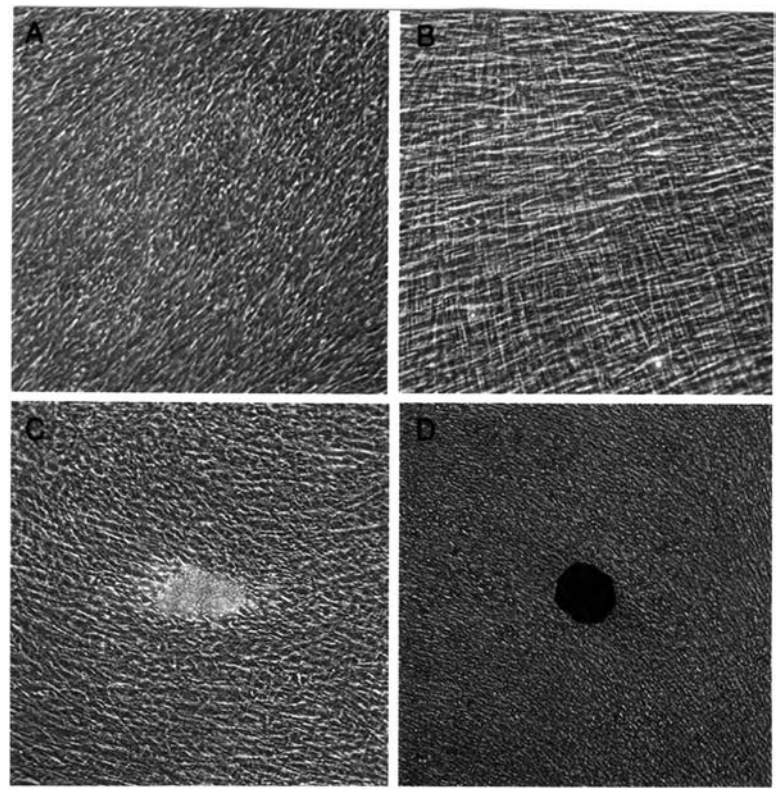

Figure 5: A photograph of 4 stages of cultured human dental pulp cells. Human dental pulp cell cultures, grown in the presence of ascorbic acid, β-glycerophosphate and fluocinolone acetonide, can be divided into four distinguished stages based on the appearance of cell alignment observed under phase contrast microscope. Four stages of cultures were identified as confluence (A), multilayer (B), nodule formation (C) and precipitation stage (D).

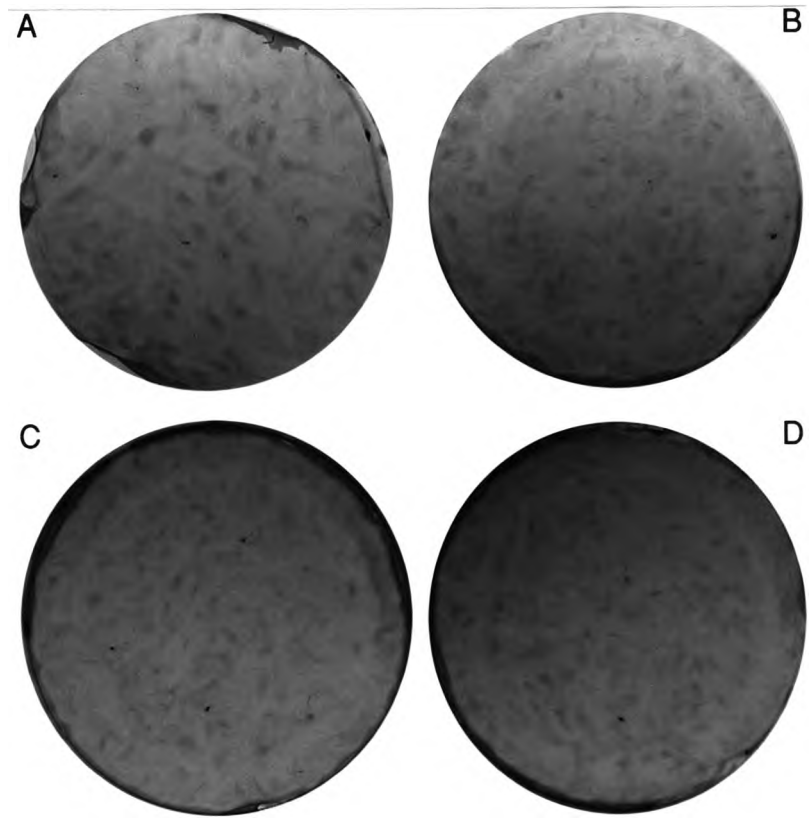

Figure 6: Photographs of calcified nodule formation demonstrated by alizarin red staining of human dental pulp cells in various concentrations of fluocinolone acetonide; Control (A), 0.1 μM (B), 1 μM (C) and 10 μM (D). The calcified nodules were macroscopically seen as red spots on the cells and seem not to differ between the experimental and control groups.