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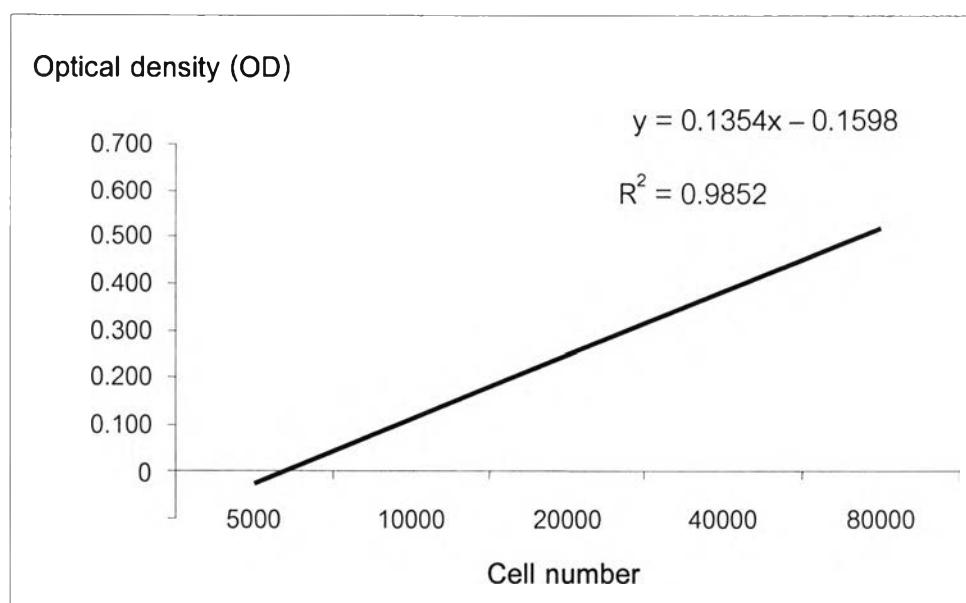
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## **APPENDICES**

**Table 2:** The effect of fluocinolone acetonide on human dental pulp cell proliferation at 24, 48 and 72 hours reported as optical density measured by MTT assay

| Concentrations of<br>fluocinolone acetonide ( $\mu\text{M}$ ) | Optical density (Mean $\pm$ S.D.) |                   |                   |
|---|-----------------------------------|-------------------|-------------------|
|   | 24 hours                          | 48 hours          | 72 hours          |
| 50  | 0.291 $\pm$ 0.024                 | 0.312 $\pm$ 0.028 | 0.317 $\pm$ 0.002 |
| 10  | 0.337 $\pm$ 0.017                 | 0.333 $\pm$ 0.020 | 0.394 $\pm$ 0.019 |
| 1   | 0.346 $\pm$ 0.025                 | 0.359 $\pm$ 0.017 | 0.419 $\pm$ 0.016 |
| 0.1   | 0.334 $\pm$ 0.024                 | 0.391 $\pm$ 0.023 | 0.460 $\pm$ 0.012 |
| Control (Serum free medium)                                   | 0.318 $\pm$ 0.008                 | 0.322 $\pm$ 0.006 | 0.352 $\pm$ 0.025 |

**Figure 7:** A standard curve of number of viable human dental pulp cells examined by MTT assay



$$\text{Number of viable cells} = \frac{(\text{O.D.} + 0.1598) \times 10^4}{0.1354}$$

$$0.1354$$

**Table 3:** The effect of fluocinolone acetonide on relative amount of type I collagen synthesis by human dental pulp cells, examined by Western blot analysis at 5 days

| គំរូ | Concentrations |         |         |         |
|------|----------------|---------|---------|---------|
|      | Control        | 0.1     | 1       | 10      |
| 1    | 100            | 151.31  | 176.76  | 184.71  |
| 2    | 100            | 125.65  | 232.79  | 202.43  |
| 3    | 100            | 136.88  | 287.83  | 196.19  |
| Mean | 100            | 137.947 | 232.460 | 194.443 |
| SD   | 0              | 7.427   | 32.064  | 5.189   |

**Table 4:** The data from Western blotting was analyzed by Shapiro-Wilk test in order to test of normality for further statistical analysis. The data of all groups were within normal distribution when the significant level was more than 0.05.

#### Tests of Normality

##### Shapiro-Wilk test

| GROUP | Statistic | df | Sig.    |
|-------|-----------|----|---------|
| 0.1   | 0.9948427 | 3  | 0.86273 |
| 1     | 0.9999735 | 3  | 0.99017 |
| 10    | 0.9716772 | 3  | 0.67705 |

**Table 5:** The data from Western blotting was tested by Levene statistic to test of homogeneity of variances between groups for further statistical analysis. The variances of data were not different when the significant level was more than 0.05.

#### **Test of Homogeneity of Variances**

WB

| Levene Statistic | df1 | df2 | Sig. |
|------------------|-----|-----|------|
| 3.002            | 3   | 8   | .095 |

**Table 6:** The data from Western blotting was analyzed by One-way ANOVA to compare means among different 3 groups. The data was statistically significant difference at the confidence level of 95%.

#### **ANOVA**

WB

|                | Sum of Squares | df | Mean Square | F      | Sig. |
|----------------|----------------|----|-------------|--------|------|
| Between Groups | 31106.29       | 3  | 10368.764   | 12.453 | .002 |
| Within Groups  | 6660.936       | 8  | 832.617     |        |      |
| Total          | 37767.23       | 11 |             |        |      |

**Table 7:** The data from Western blotting was analyzed by Scheffe test to perform all pairwise comparisons between 2 different groups. The data was statistically significant difference when the significant level was less than 0.05. The results showed that group 3 (1  $\mu$ M) and 4 (10  $\mu$ M) were significant different from group 1 (control), and group 2 (0.1  $\mu$ M) was significant different from group 3.

### Multiple Comparisons

Dependent Variable: WB  
Scheffe

| (I) GROUP | (J) GROUP | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval |             |
|-----------|-----------|-----------------------|------------|------|-------------------------|-------------|
|           |           |                       |            |      | Lower Bound             | Upper Bound |
| 1         | 2         | -37.9467              | 23.5601    | .498 | -120.2336               | 44.3403     |
|           | 3         | -132.4600*            | 23.5601    | .004 | -214.7469               | -50.1731    |
|           | 4         | -94.4433*             | 23.5601    | .026 | -176.7303               | -12.1564    |
| 2         | 1         | 37.9467               | 23.5601    | .498 | -44.3403                | 120.2336    |
|           | 3         | -94.5133*             | 23.5601    | .026 | -176.8003               | -12.2264    |
|           | 4         | -56.4967              | 23.5601    | .205 | -138.7836               | 25.7903     |
| 3         | 1         | 132.4600*             | 23.5601    | .004 | 50.1731                 | 214.7469    |
|           | 2         | 94.5133*              | 23.5601    | .026 | 12.2264                 | 176.8003    |
|           | 4         | 38.0167               | 23.5601    | .496 | -44.2703                | 120.3036    |
| 4         | 1         | 94.4433*              | 23.5601    | .026 | 12.1564                 | 176.7303    |
|           | 2         | 56.4967               | 23.5601    | .205 | -25.7903                | 138.7836    |
|           | 3         | -38.0167              | 23.5601    | .496 | -120.3036               | 44.2703     |

\*. The mean difference is significant at the .05 level.

**Table 8:** The effect of fluocinolone acetonide on relative amount of type I collagen mRNA expression by human dental pulp cells, examined by RT-PCR at 48 hours

| ครั้งที่ | RT-PCR  |        |
|----------|---------|--------|
|          | Control | 1 µM   |
| 1        | 100     | 302.84 |
| 2        | 100     | 313.79 |
| 3        | 100     | 239.03 |
| Mean     | 100.00  | 285.22 |
| SD       | 0.000   | 40.375 |

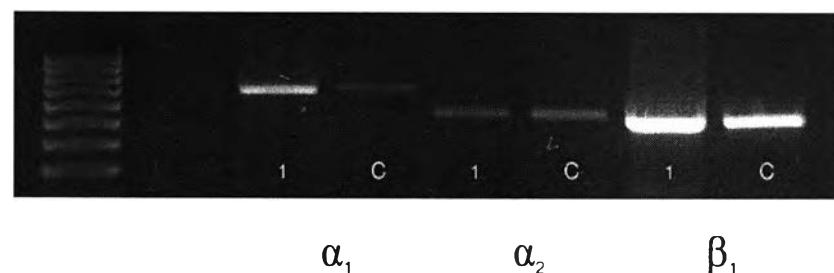
**Table 9:** The data from RT-PCR was analyzed by Shapiro-Wilk test in order to test of normality for further statistical analysis. The data was within normal distribution when the significant level was more than 0.05.

| Tests of Normality |           |    |         |
|--------------------|-----------|----|---------|
| Shapiro-Wilk test  |           |    |         |
| RT                 | Statistic | df | Sig.    |
| 1 µM               | 0.8571582 | 3  | 0.25979 |

**Table 10:** The data from RT-PCR was analyzed by t test to perform pair wise comparison between 2 different groups. The results showed statistically significant difference between 1 µM fluocinolone acetonide and control groups.

| Independent Samples Test |  |                  |                              |            |                 |                        |                       |   |                       |
|--------------------------|--|------------------|------------------------------|------------|-----------------|------------------------|-----------------------|---|-----------------------|
|                          | Levene's Test for Equality of Variances                |                  | t-test for Equality of Means |            |                 |                        |                       |   |                       |
|                          | F  | Sig.             | t                            | df         | Sig. (2-tailed) | Mean Difference        | Std. Error Difference | 95% Confidence Interval of the Difference |                       |
|                          |  |                  |                              |            |                 |                        |                       | Lower                                     | Upper                 |
| RT                       | Equal variances assumed<br>Equal variances not assumed | 13.692<br>-7.946 | .021<br>-7.946               | 4<br>2.000 | .001<br>.015    | -185.2200<br>-185.2200 | 23.3103               | -249.9398<br>-285.5162                    | -120.5002<br>-84.9238 |

**Figure 8:** The expression of integrin receptors of human dental pulp cells at 48 hours cultured with 1  $\mu$ M fluocinolone acetonide. The expression of  $\alpha_1$  and  $\beta_1$  integrin receptors seem to higher than in control. (1 = 1  $\mu$ M fluocinolone acetonide, C = control,  $\alpha_1$  =  $\alpha_1$  integrin receptor,  $\alpha_2$  =  $\alpha_2$  integrin receptor,  $\beta_1$  =  $\beta_1$  integrin receptor)



## Author's Biography

Mr. Phumisak Louwakul was born in August 25, 1976 in Bangkok, Thailand. He graduated from Faculty of Dentistry, Chiang Mai University in 2000. He worked at Sirindhorn College of Public Health, Trang from 2000 to 2001, and the Department of Restorative Dentistry, Faculty of Dentistry, Chiang Mai University from 2002 until now. By the grant of Co-operative Research Network (CRN), he started his postgraduate study for the Master Degree of Science in Endodontontology, Faculty of Dentistry, Chulalongkorn University.

