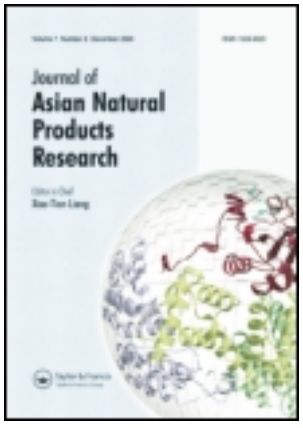

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

In vitro study of the effects of plaunotol on oral cell proliferation and wound healing

Siribang-on Piboonniyom Khovidhunkit [a], Nadiya Yingsaman [b], Kongtawat Chairachvit [c], Rudee Surarit [c], Pornpoj Fuangtharnthip [a] & Amorn Petsom [d]

[a] Department of Hospital Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, 10400, Thailand

[b] Chainat Hospital, Chainat, 17000, Thailand

[c] Department of Physiology and Biochemistry, Faculty of Dentistry, Mahidol University, Bangkok, 10400, Thailand

[d] Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok, 10330, Thailand

Available online: 28 Jan 2011

To cite this article: Siribang-on Piboonniyom Khovidhunkit, Nadiya Yingsaman, Kongtawat Chairachvit, Rudee Surarit, Pornpoj Fuangtharnthip & Amorn Petsom (2011): *In vitro* study of the effects of plaunotol on oral cell proliferation and wound healing, Journal of Asian Natural Products Research, 13:02, 149-159

To link to this article: http://dx.doi.org/10.1080/10286020.2010.546790

Journal of Asian Natural Products Research
Vol. 13, No. 2, February 2011, 149–159

In vitro study of the effects of plaunotol on oral cell proliferation and wound healing

Siribang-on Piboonniyom Khovidhunkit[a]*, Nadiya Yingsaman[b], Kongtawat Chairachvit[c], Rudee Surarit[c], Pornpoj Fuangtharnthip[a] and Amorn Petsom[d]

[a]*Department of Hospital Dentistry, Faculty of Dentistry, Mahidol University, Bangkok 10400, Thailand;* [b]*Chainat Hospital, Chainat 17000, Thailand;* [c]*Department of Physiology and Biochemistry, Faculty of Dentistry, Mahidol University, Bangkok 10400, Thailand;* [d]*Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*

(*Received 21 August 2010; final version received 7 December 2010*)

Plaunotol is an acyclic diterpene alcohol extracted from a medicinal plant called plau-noi, *Croton stellatopilosus* Ohba, and has been widely used for the treatment of gastric ulcers in Japan. The aim of this study was to examine the effects of plaunotol on human gingival fibroblasts (HGFs) and human oral keratinocytes (HOKs). To assess the cytotoxic effect, HGFs and HOKs were treated with plaunotol. Subsequently, the morphology of cells was recorded and cells were subjected to MTT assay. To investigate cell proliferation effect, cells were treated with plaunotol and counted with a haemocytometer. To determine wound healing effect, the number of cells repopulated into the wounded areas in monolayer culture and in fibroblast-populated collagen lattice (FPCL) was measured. The results showed that 10 and 1 μg/ml (33 and 3.3 μmol/l) plaunotol induced toxicity in HGFs and HOKs, respectively. However, 0.1 μg/ml (0.33 μmol/l) plaunotol promoted HGF proliferation and wound healing in monolayer and FPCL models. In contrast, 0.1 μg/ml plaunotol could not induce HOK proliferation nor *in vitro* wound healing using monolayer culture, but it induced wound healing in a modified FPCL model. Our data suggested that plaunotol could promote oral cell proliferation and wound healing *in vitro* and may have an implication on oral wound healing.

Keywords: plaunotol; *Croton stellatopilosus* Ohba; oral human gingival fibroblasts; oral human keratinocytes; oral wound healing

1. Introduction

Plaunotol (Figure 1) [1] is an acyclic diterpene alcohol extracted from *Croton stellatopilosus* Ohba, a medicinal plant called plau-noi found mainly in Southeast Asia and certain provinces of Thailand. In Japan, plaunotol (**1**) was developed into a commercial drug named Kelnec™. This drug has been used for the treatment of gastric ulcer and gastritis for over 10 years, because of its strong anti-ulcer activities and low toxicity [2]. It has been reported that **1** can promote the healing of gastric ulcer first by reducing gastric acid secretion [3] and second by eradicating *Helicobacter pylori (H. pylori)* [1,4]. Since **1** could promote gastric ulcer healing, it should be interesting to investigate its effect on the treatment of oral ulcers.

An oral ulcer is a break in the lining of the mouth that uncovers the sensitive tissue beneath. Two common and painful types of ulcers are the recurrent aphthous ulcers (RAUs) and traumatic ulcers. RAUs, or

*Corresponding author. Email: dtspb@mahidol.ac.th

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2010.546790
http://www.informaworld.com

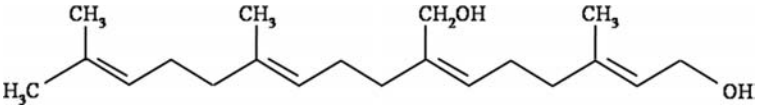

Figure 1. Chemical structure of plaunotol (**1**) [1].

canker sores, are among the oral mucosal conditions that dentists most commonly encounter. There are three clinical forms of RAUs: RAU minor, RAU major, and herpetiform RAU [5]. The etiology of RAU is currently unknown but several local, systemic, immunologic, genetic, allergic, nutritional, and microbial factors have been proposed as causative agents [6]. *H. pylori* may have a causative role in RAUs and can be detected in oral ulcers in apparently immunocompetent adults, however, it is inconclusive whether these microorganisms are in fact the cause of RAUs [7]. Traumatic ulcers result from injuries involving the oral cavity which can be either acute or chronic. Traumatic ulcers can occur at any location and may have variable features depending on their causes, either mechanical, thermal, or chemical.

Immunomodulating agents especially topical steroids have been the mainstay of treatment for oral ulcers [8]. Since steroids suppress the immune response, the major disadvantage of steroids is opportunistic infections especially oral candidiasis [9]. Alternative agents that promote wound healing of the oral cavity by mechanisms different from those of steroids may be of clinical interest. The objective of this study was to evaluate the *in vitro* effects of **1** on human gingival fibroblasts (HGFs) and oral epithelial cells using tissue culture-based experiments. The result of this study may lead to an alternative herbal medication for the treatment of oral ulcer.

2. Results and discussion

2.1 Cytotoxic effect of 1 on HGFs and HOKs

To investigate the cytotoxicity of **1** on HGFs, cells were exposed to **1** at various concentrations for 48 h. The morphology of cells treated with 1–10 μg/ml of **1** was similar to that of the control, however, in the group treated with 25 μg/ml of **1**, disruption of the cell membrane and detachment of cells were appreciated (Figure 2(A)). To confirm the cytotoxicity of **1**, an MTT assay was used (Figure 2(B)). There was no significant difference of percent viability observed from cells treated with 1–10 μg/ml of **1** both at 24 and 48 h compared to that of control. The result regarding the morphological changes was relevant to the MTT part in that no change of cell morphology was observed when cells were treated with 1–10 μg/ml of **1**. In human oral keratinocytes (HOKs), the morphology of cells treated with 1 μg/ml of **1** was similar to that of control. However, in the groups treated with more than 1 μg/ml of **1**, a lot of cell death and detachment of cells were noted (Figure 3(A)). According to MTT assay, the viability of cells was decreased in the **1**-treated groups in a dose-dependent manner (Figure 3(B)). In conclusion, HOKs appeared to be more sensitive to the cytotoxic effect of **1** than HGFs.

This result implied that the effects of **1** may be cell-type specific. This substance has been found to induce apoptosis in three gastric cancer cell lines, namely MKN-45, MKN-74, and AZ-521 [10]. In this report, **1** at the concentrations of 10, 20, 30, and 40 μmol/l was used. It was found that **1** dose dependently inhibited the growth of all gastric cancer cells, dependent on the induction of apoptosis. Caspases-8, -9, and -3 were found to be activated in the apoptotic cells, and the expression of Bax protein was increased. In another study, a colon cancer cell line, DLD1, was cultured

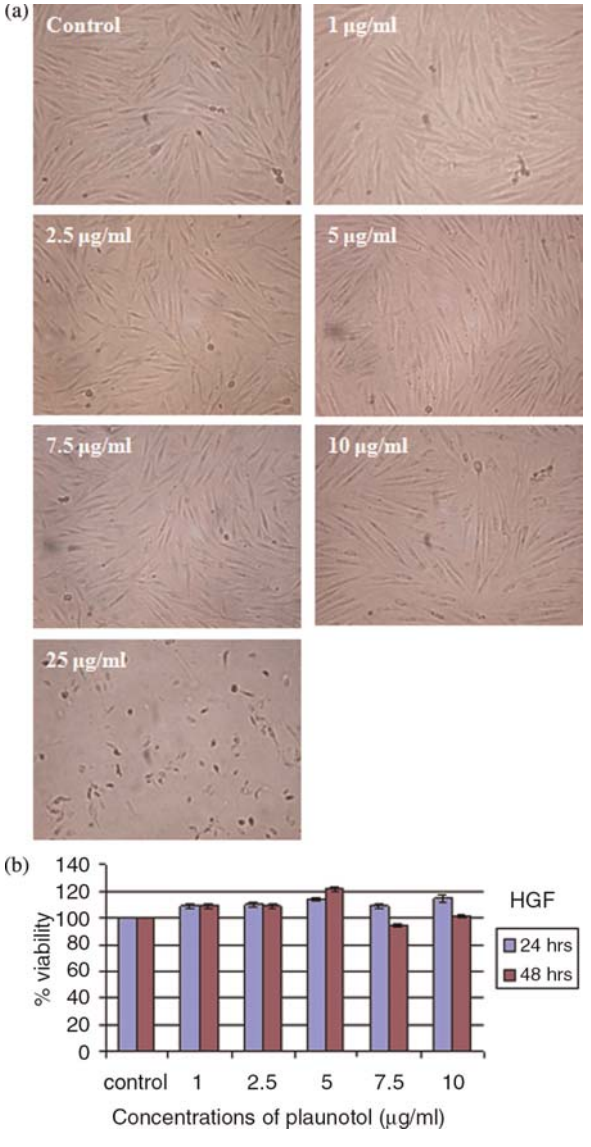

Figure 2. Cytotoxic effect of **1** on HGFs. (A) Optical micrographs of HGFs after treatment with various concentrations of **1** for 48 h. (B) Percent viability of HGFs after the treatment.

in the presence of **1** and its proliferation was measured by MTS assay [11]. The result showed that **1** strongly inhibited the proliferative activity of DLD1, dependent on the induction of apoptosis. Although in this present study, the apoptosis of HOKs was not investigated, it should be interesting to further investigate the molecular mechanism of **1** to induce cell death of HOKs in the future.

2.2 Proliferative effect of 1 on HGFs and HOKs

To determine the proliferative effect of **1**, cells were treated with **1** for up to 72 h at concentrations lower than 1 μg/ml to avoid the cytotoxic effect, and the cell number in each group was counted. For HGFs, proliferation was significantly increased at each time point when incubated with 0.1 μg/ml of **1** (Figure

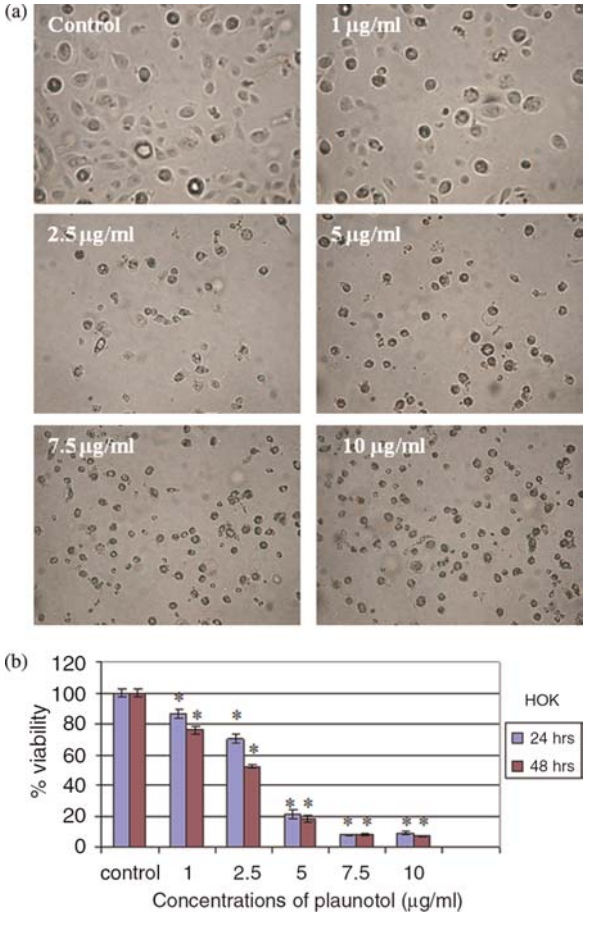

Figure 3. Cytotoxic effect of **1** on HOKs. (A) Optical micrographs of HOKs after treatment with various concentrations of **1** for 48 h. (B) Percent viability of HOKs after the treatment. A significant difference is shown with an asterisk (*) at $p < 0.05$ when compared with the control group.

4(A)). However, higher concentrations of **1** (0.25–1 μg/ml) did not affect HGF proliferation. On the contrary, it was found that **1** (0.1–1 μg/ml) did not induce HOK proliferation (Figure 4(B)). In fact, a significant reduction in HOK number was observed at higher concentrations, suggesting that **1** was more toxic to HOKs compared to HGFs.

These results suggested that a low dose (0.1 μg/ml) of **1** could promote proliferation of HGFs, but not HOKs, under unstimulated conditions. In a study of endothelial cells, **1** has been shown to exhibit anti-proliferative effect in a dose-dependent manner. In addition, **1** was found to inhibit the ability of endothelial cells to form tube-like structures in matrigel due to the selective suppression of $\alpha_v\beta_3$ function and not by direct induction of apoptosis [12]. Therefore, it indicated that the effects of **1** are cell type specific and treatment of **1** on different cell-types may give rise to different results.

2.3 Effect of 1 on wound healing using HGF and HOK monolayer cultures

To examine whether **1** could induce monolayer wound healing, a confluent

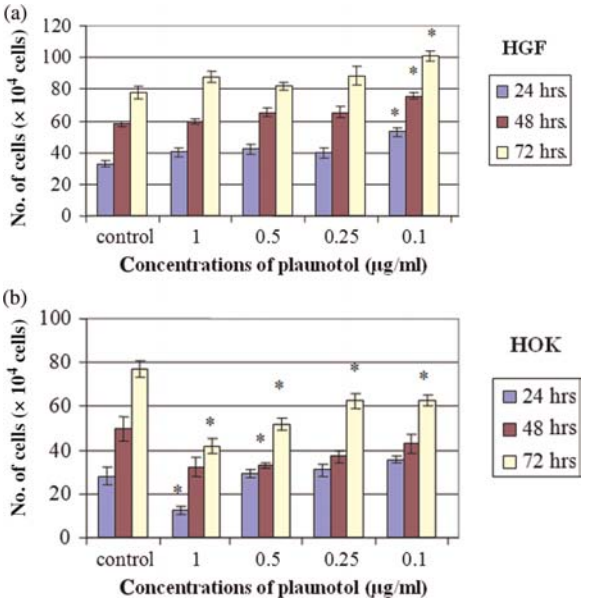

Figure 4. Proliferative effect of **1** on HGFs and HOKs. (A) The number of HGFs after incubation with **1** for 24, 48, and 72 h. (B) The number of HOKs after incubation with **1** for 24, 48, and 72 h. A significant difference is shown with an asterisk (*) at $p < 0.05$ when compared with the control group.

monolayer of cells was scraped and incubated with **1**. Incubation of HGFs with 0.1 μg/ml of **1** resulted in significant repopulation of cells (approximately two fold compared to that of the control group) into the wounded area (Figure 5(A) and (B)). Incubation with higher concentrations of **1** did not result in the significantly different cell number repopulation. Incubation of HOKs with **1** (0.1–1 μg/ml) did not result in a statistically significant difference in the cell number repopulated into the wounded areas compared to that of control (Figure 6(A) and (B)). Collectively, these results indicated that a lower concentration of **1** could promote monolayer wound healing using HGFs but not HOKs.

2.4 *Effect of 1 in 3D* in vitro *wound healing*

To provide a 3D system and to study the oral mucosal wound repair, multiple fibroblast-populated collagen lattices (FPCLs) were created [13]. In agreement with the results using the monolayer culture, it was found that 0.1 μg/ml of **1** induced wound healing in a 3D FPCL model (Figure 7(A)). The number of HGFs repopulated into the wounded area was approximately two fold compared to that of the control group (Figure 7(A) and (B)). To examine the effect of **1** on HOKs in *in vitro* wound healing, multiple FPCL were fabricated except that HOKs were laid down on the FPCL, then wounded by a punch biopsy. This model is called epidermal equivalent model. It was found that on the first day after treatment with **1** or 0.5 ng/ml epidermal growth factor (EGF), foci of HOKs were present in the wounded defect. On the second day, sheaths of an epithelium-like structure were observed in the 0.1 μg/ml of **1**-treated and the EGF-treated groups. On day 4, HOKs epithelialized to cover approximately three quarters of the wounded defect only in the 0.1 μg/ml

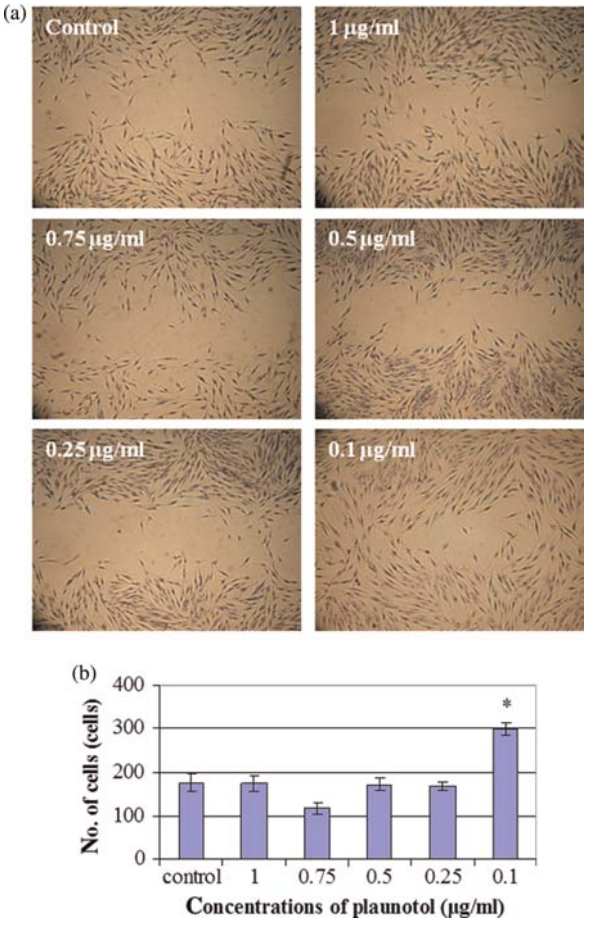

Figure 5. Effect of **1** on wound healing using HGF monolayer culture. (A) Micrographs of HGFs after incubation with **1** for 24 h. (B) The number of cells repopulating the wound space. An asterisk (*) indicates a significant difference in the number of cells compared to that of control.

of **1**-treated and the EGF-treated groups. Only a few foci of HOKs were noted in the control and the 0.5 µg/ml of **1**-treated groups (Figure 8(A) and (B)). When the number of HOKs was compared, it was found that the number of HOKs repopulated into the wounded area in the 0.1 µg/ml of **1**-treated group was approximately 20-fold compared to that of the control group. This result suggested that 0.1 µg/ml of **1** promoted HOK repopulation in the epidermal equivalent model. Since the main objective of our present study was to determine the effect of **1** on *in vitro* wound healing, we used both monolayer culture and FPCL models. In our study, **1** promoted HGF cell migration both in the monolayer and the FPCL system. For HOKs, however, **1** did not promote cell migration in a monolayer culture, but it promoted re-epithelialization in an epidermal equivalent FPCL model in the same manner as EGF.

Although simple monolayer cultures have been utilized as models for initial screening to examine an appropriate concentration of the chemical to induce wound repair, they allow the study of

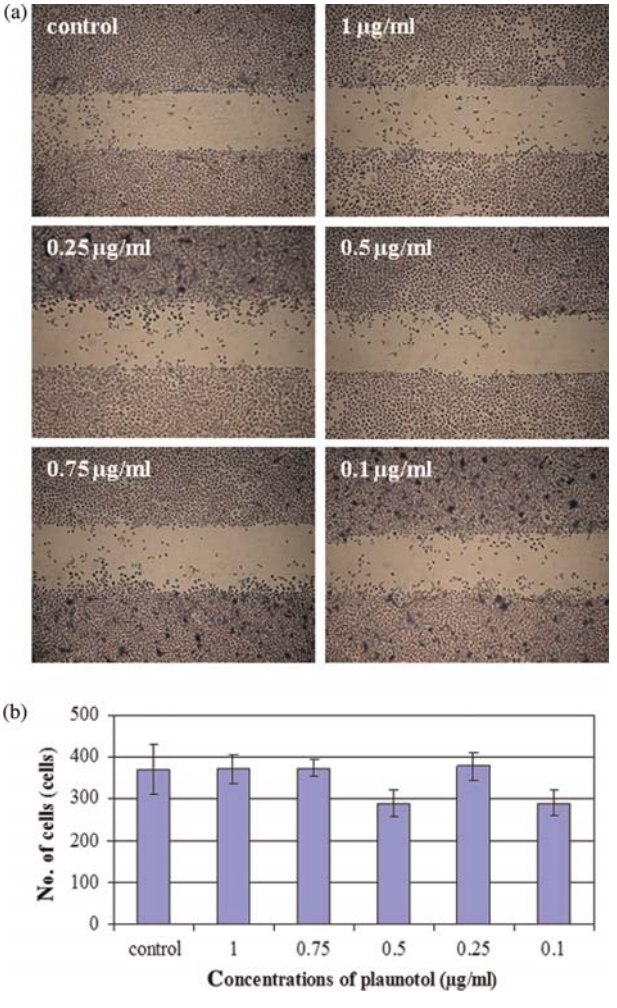

Figure 6. Effect of **1** on wound healing using HOK monolayer culture. (A) Micrographs of HOKs after incubation with **1** for 24 h. (B) The number of cells repopulating the wound space.

a limited number of aspects. Monolayer cultures therefore cannot be used to replicate the interactions of the cells with its extracellular matrix or other cell types. In addition, cells grown on a plastic substratum *in vitro* are densely packed with many cell–cell interactions with very little extracellular matrix present [14]. Therefore, the FPCL system is a useful 3D model to isolate and study the aspects of cutaneous wound healing. In our study, approximately two fold fibroblasts repopulated into the wounded defect were significantly stimulated in the presence of 0.1 μg/ml of **1** and EGF compared to control. This is in agreement with the work done by Genever *et al.* [15] who found a 2.8-fold increase in HGF migration when cells were treated with EGF. For HGFs, **1** promoted cell migration both in the monolayer and FPCL. For HOKs, we found that **1** did not promote cell repopulation in wound created on monolayer. However, in wounding epidermal equivalent, **1** promoted re-epithelialization in the same manner as EGF. Since interaction

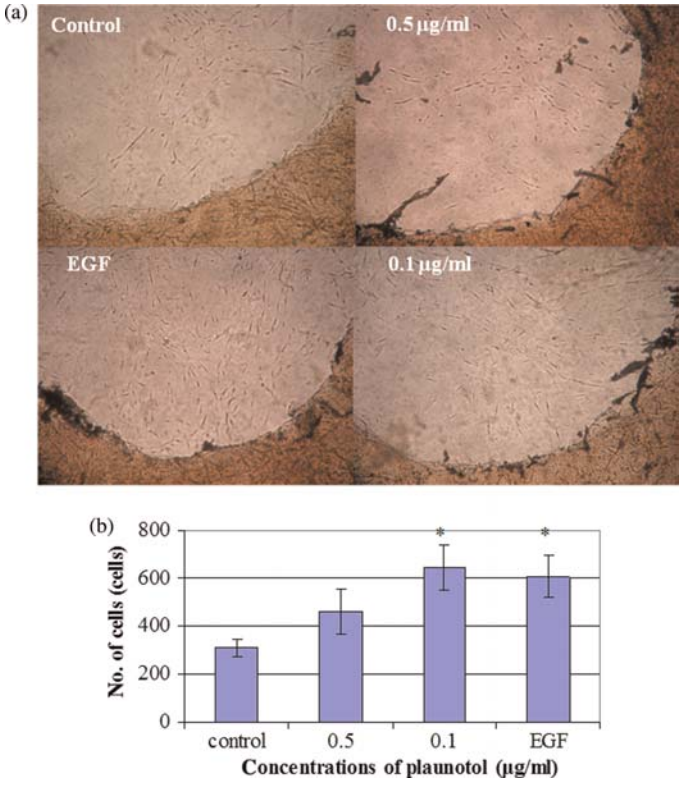

Figure 7. Effect of **1** on HGFs in a 3D *in vitro* wound healing model. (A) Micrographs of HGF repopulation of the wounded FPCL 3 days after wounding. (B) The number of cells repopulating the wounded space. An astrisk (*) indicates a significant difference in the number of cells compared to that of control.

between cells and extracellular matrix may induce wound healing process more efficiently than cells and plastic substratum [16], we assume that the result from epidermal equivalent model would represent more physiological situation than that in the monolayer cell culture and believed that 0.1 µg/ml of **1** could promote HOK proliferation and migration.

In summary, our study showed that 0.1 µg/ml of **1** could promote HGF proliferation and wound healing in both 2D- and 3D models *in vitro*. In addition, **1** could also promote wound healing using HOKs in the 3D model. These results suggest that low dose of **1** is effective in oral mucosal wound healing *in vitro* and further investigations *in vivo*, such as in animal models, are needed to confirm our findings. This substance may have a clinical implication on oral wound healing and may emerge as an effective alternative agent for the treatment of oral ulcers.

3. Experimental

3.1 Cell culture

HGFs were derived from gingiva received from gingival surgery. Cells were seeded and cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (Gibco™, Invitrogen Corporation, Carlsbad, CA, USA) at 37°C in humidified atmosphere of 95% air and 5% CO_2. The HOKs were established by Piboonniyom *et al.* [17]. These cells were immortalized HOKs using human telomer-

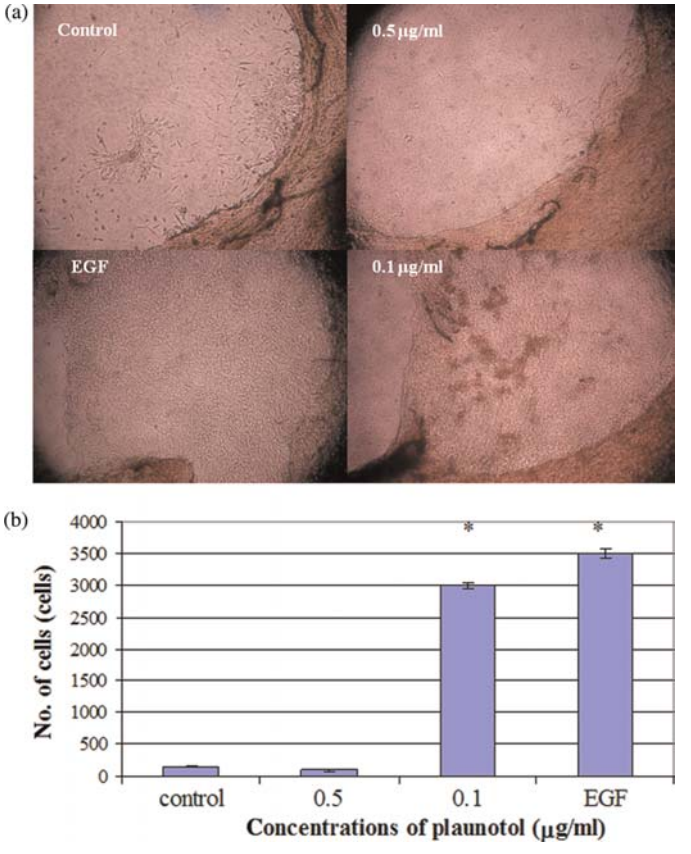

Figure 8. Effect of **1** on HOKs in a 3D *in vitro* wound healing method. (A) Micrographs of HOK repopulation of the wounded FPCL 4 days after wounding. (B) The number of cells repopulating the wounded space. An asterisk (*) indicates a significant difference in the number of cells compared to that of control.

ase reverse transcriptase (*h-TERT*, a kind gift from R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, MA, USA) and cdk4. HOKs were grown in keratinocyte serum-free medium (Keratinocyte-SFM, GIBCO™, Invitrogen Corporation) supplemented with EGF and bovine pituitary extract.

3.2 Cytotoxic assay

3.2.1 Morphological observation

HGFs were added to each well of six-well culture plates (Costar™, Sigma-Aldrich, St. Louise, MO, USA) at 1×10^5 cells. For HOKs, 2×10^5 cells per well were used. Cells were seeded and incubated at 37°C in humidified atmosphere and 5% CO_2 for 24 h prior to the treatment. Solutions of **1** were prepared by diluting **1** (a kind gift from Dr A. Petsom, Faculty of Science, Chulalongkorn University, Thailand) in DMSO. The non-confluent HGFs were treated with **1** at concentrations of 1, 2.5, 5, 7.5, 10, and 25 μg/ml (equivalent to 3.3, 8.25, 16.5, 24.75, 33, and 82.5 μmol/l, respectively) for 48 h. For HOKs, cells were treated with **1** at concentrations of 1, 2.5, 5, 7.5 and 10 μg/ml (equivalent to 3.3, 8.25, 16.5, 24.75 and 33 μmol/l, respectively) for 48 h. In the control group, cells were treated with DMSO alone.

At each concentration, cell morphology was examined under light microscope and photographs were taken by a digital camera (Nikon Coolpix 990, Tokyo, Japan).

3.2.2 *MTT assay*

The method of colorimetric cytotoxic assay described by Kasugai *et al.* [18] was used with some modifications. In brief, HGFs or HOKs were added to each well of 96-well culture plates (Costar™) at a concentration of 2×10^4 cells in 100 μl medium per well, then treated with 1, 2.5, 5, 7.5, and 10 μg/ml (equivalent to 3.3, 8.25, 16.5, 24.75, and 33 μmol/l, respectively) of **1**. For control group, cells were treated with DMSO alone. After 24 or 48 h of incubation, 10 μl of the MTT solution (5 mg/ml prepared in media) was added and incubated for 3 h. The entire medium was then removed and washed with PBS twice. The wells were then filled with 100 μl DMSO and agitated. The spectrophotometric absorbance at 540 nm was then measured by an ELISA reader (Ceres UV 900 HDi, BioTek Instrument, Winooski, VT, USA) with DMSO as blank. The mitochondrial dehydrogenase activity of cells at each **1** concentration was calculated as a percentage of the control activity from the absorbance values. In the control group, the percent viability was set as 100%. The percent viability in the **1**-treated groups was calculated by dividing the absorbance value of each group by the control absorbance value multiplied by 100.

3.3 *Cell proliferation*

HGFs were added to each well of 6-well culture plates (Costar™) at 1×10^5 cells per well. For HOKs, 2×10^5 cells per well were used. Cells were then treated with 0.1, 0.25, 0.5, 0.75, and 1 μg/ml (equivalent to 0.33, 0.825, 1.65, 2.475, and 3.3 μmol/l, respectively) of **1** for 24, 48, and 72 h. In the control group, cells were treated with DMSO alone. Cells were trypsinized with 0.1% trypsin solution (GIBCO™, Invitrogen Corporation) and counted under a light microscope at each concentration.

3.4 *Wound healing on monolayer culture*

A confluent monolayer of HGFs or HOKs was cultured on 6-cm dishes, then a wound was created in the cultured cells by scraping a plastic pipette tip from the center of the well extending to the edge across the surface of tissue culture well [19]. Cells were subsequently incubated with **1** at concentrations of 0.1, 0.25, 0.5, 0.75, and 1 μg/ml (equivalent to 0.33, 0.825, 1.65, 2.475, and 3.3 μmol/l, respectively) and DMSO was used as control. Cells were further incubated for 24 h and repopulating cells in the wounded areas were fixed and stained with toluidine blue. Wound repopulation images were acquired using six low power (4 ×) areas along the length of the wound for each experimental well. Wound repopulation was determined by measurement of the cell number.

3.5 **In vitro** *wound healing assay*

An *in vitro* wound healing assay was established as previously described [20]. Type I collagen was extracted and purified from rat tail tendon according to O'Leary *et al.* [21]. Cultured HGFs were combined with type I collagen in nutrient medium to form the FPCL. For 'epidermal' equivalent, multiple FPCLs were fabricated in the same manner except that HOKs were laid down on the FPCL. The FPCL was allowed to contract for 7 days until it reached approximately 10–30% of the initial diameter. The lattices were wounded by a 4-mm diameter punch biopsy. Immediately after wounding, the lattice was transferred to an acellular collagen lattice and 10 μl of collagen solution was applied to the collagen lattice to act as glue between the

two lattices. These lattices were then incubated with **1** at concentrations of 0.1 or 0.5 μg/ml and DMSO or 0.5 ng/ml EGF were used as controls. Three days after wounding, cells were photographed under a light microscope for subsequent counting of the number of cells repopulating the wounded space.

3.6 Statistical analysis

Each experiment was performed three times, and the mean and the standard deviation were calculated. Data were tested for normal distribution by the Kolmogorov–Smirnov method and equal variances by the Levene test. One-way analysis of variance and Scheffe's method were used when data indicated normal distribution and equal variance. Otherwise, data were analyzed by non-parametric tests. Kruskal–Wallis ANOVA on ranks and Mann–Whitney test were used to determine the significance of differences between groups. A p-value less than 0.05 was considered statistically significant.

References

[1] T. Koga, H. Kawada, Y. Utsui, H. Domon, C. Ishii, and H. Yasuda, *J. Antimicrob. Chemother.* **38**, 387 (1996).

[2] T. Takeuchi, K. Shiratori, S. Watanabe, J.H. Chang, Y. Moriyoshi, and K. Shimizu, *J. Clin. Gastroenterol.* **13** (Suppl 1), S83 (1991).

[3] H. Kaneko, T. Mitsuma, H. Nagai, M. Harada, H. Kotera, A. Furusawa, and K. Morise, *Dig. Dis. Sci.* **40**, 160 (1995).

[4] T. Koga, H. Kawada, Y. Utsui, H. Domon, C. Ishii, and H. Yasuda, *J. Antimicrob. Chemother.* **37**, 919 (1996).

[5] S.O. Akintoye and M.S. Greenberg, *Dent. Clin. North. Am.* **49**, 31 (2005).

[6] S.B. Woo and S.T. Sonis, *J. Am. Dent. Assoc.* **127**, 1202 (1996).

[7] S.R. Porter, G.R. Barker, C. Scully, G. Macfarlane, and L. Bain, *Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod.* **83**, 325 (1997).

[8] S.D. Vincent and G.E. Lilly, *Oral. Surg. Oral. Med. Oral. Pathol.* **74**, 79 (1992).

[9] F. Lozada, S. Silverman, and C. Migliorati, *J. Am. Dent. Assoc.* **109**, 269 (1984).

[10] J. Yamada, K. Kawai, N.H. Tsuno, J. Kitayama, T. Tsuchiya, S. Yoneyama, M. Asakage, Y. Okaji, K. Takahashi, and H. Nagawa, *Planta Med.* **73**, 1068 (2007).

[11] N. Yoshikawa, J. Yamada, N.H. Tsuno, Y. Okaji, K. Kawai, T. Tsuchiya, S. Yoneyama, J. Tanaka, Y. Shuno, T. Nishikawa, H. Nagawa, N. Oshima, and K. Takahashi, *J. Surg. Res.* **153**, 246 (2009).

[12] K. Kawai, N.H. Tsuno, J. Kitayama, Y. Okaji, K. Yazawa, M. Asakage, H. Yamashita, T. Watanabe, K. Takahashi, and H. Nagawa, *Anticancer Drugs* **16**, 401 (2005).

[13] R. O'Leary and E.J. Wood, In Vitro *Cell Dev. Biol. Anim.* **39**, 204 (2003).

[14] G.I. Howling, P.W. Dettmar, P.A. Goddard, F.C. Hampson, M. Dornish, and E.J. Wood, *Biotechnol. Appl. Biochem.* **36**, 247 (2002).

[15] P.G. Genever, E.J. Wood, and W.J. Cunliffe, *Exp. Dermatol.* **2**, 266 (1993).

[16] P. Stephens, E.J. Wood, and M.J. Raxworthy, *Wound Repair Regen.* **4**, 393 (1996).

[17] S.O. Piboonniyom, S. Timmermann, P. Hinds, and K. Munger, *Oral Oncol.* **38**, 179 (2002).

[18] S. Kasugai, N. Hasegawa, and H. Ogura, *Jpn. J. Pharmacol.* **52**, 95 (1990).

[19] M. Tomic-Canic, S.W. Mamber, O. Stojadinovic, B. Lee, N. Radoja, and J. McMichael, *Wound Repair Regen.* **15**, 71 (2007).

[20] E. Bell, B. Ivarsson, and C. Merrill, *Proc. Natl. Acad. Sci. USA* **76**, 1274 (1979).

[21] R. O'Leary, M. Arrowsmith, and E.J. Wood, *Cell Biochem. Funct.* **20**, 129 (2002).