

The Role of Prostaglandin E₂ on Osteoblast Proliferation Induced by Hydroxyapatite

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Abstract

Objective: Prostaglandin E₂ (PGE₂) plays a crucial role in regulating bone cell differentiation and proliferation. The aim of the present study was to determine whether PGE₂ may regulate osteoblast proliferation induced by hydroxyapatite. **Materials and Methods:** Osteoblasts (HOS cell line) pre-treated with cyclooxygenase (COX) inhibitors (indomethacin, aspirin and nimesulide) were then cultured. The cells were also pre-treated with or without nimesulide and then cultured with PGE₂. The cell cultures were also treated with SQ22536 (adenylyl cyclase inhibitor) and added with Db-cAMP (cAMP analog), and/or PGE₂. KT5720 [protein kinase A (PKA) inhibitor], Db-cAMP and/or forskolin (adenylyl cyclase activator)-treated cultures were used to assess the role of PKA. The role of EP2 and/or EP4 was determined by using EP2 antagonist (PF-04418948) and EP4 antagonist (L-161,982) with PGE₂. All cells were cultured with or without hydroxyapatite. The levels of PGE₂ and cAMP were detected from the culture supernatants and the cell proliferation was assessed colorimetrically. **Results:** Nimesulide and indomethacin but not aspirin suppressed partially the cell proliferation but fully PGE₂ production. PGE₂ abrogated nimesulide-mediated suppression of cell proliferation. The cell proliferation was enhanced by low but suppressed by high concentration of PGE₂. Moreover, the SQ22536-mediated suppression of cell proliferation was abolished by Db-cAMP but not PGE₂. Conversely, PGE₂, Db-cAMP or forskolin failed to eliminate KT5720-mediated suppression of cell proliferation. The effect of PGE₂ on cell proliferation and cAMP levels was mediated predominantly via EP2 and to a lesser extent, EP4. The results of the controls for all experiments were significantly lower than hydroxyapatite-stimulated cell cultures. **Conclusion:** These results suggest that PGE₂, acting via a COX-2-, cAMP-PKA- and both EP2 and EP4-dependent pathway may partially regulate hydroxyapatite-induced human osteoblasts in an autocrine fashion.

Keywords

Hydroxyapatite, Osteoblast, PGE₂, Proliferation

1. Introduction

Prostaglandin E₂ (PGE₂), a prostanoid produced by the action of cyclooxygenases (COX-1 and COX-2) on arachidonic acid, is known as a potent regulator on bone remodeling, since it has ability to activate osteoclast and osteoblast differentiation and proliferation by binding with its receptors, *i.e.*, EP1, EP2, EP3, and EP4 [1]. It seems plausible that all four PGE₂ receptors may play a regulatory role in the dynamic bone remodeling. EP2 and/or EP4 bound by PGE₂ or its analogs are shown to be the receptors that activate the cAMP-PKA pathway leading to COX-2 activities and bone formation [2] [3]. On the other hand, binding PGE₂ on cell surface EP1 or EP3 may result in the reduction of bone formation [3] [4], although others demonstrated that EP2 and/or EP4 may also play a role in bone resorption [4] [5].

Bone remodeling at the site of implantation seems also to be under regulation of PGE₂. Regardless of the implant surface topography, PGE₂ suppressed the conical Wnt signaling pathway of osteoblasts leading to downregulation of cell differentiation on the surface of implants [6]. However, others indicated that PGE₂ production by osteoblasts plated on the rough surface is lower than that by cells plated on the smooth surface and thus, the cell number and differentiation are increased on the former surface [7] [8], suggesting that the regulatory role of PGE₂ on implant-induced osteoblast differentiation and proliferation may be dependent on the implant surface microstructure. The effect of PGE₂ on bone remodeling may also be dependent on the concentration of this cytokine. This notion was based on the fact that low and high concentration of PGE₂ induce bone formation and destruction in the implanted site, respectively [9].

Potent osteoconductive properties of hydroxyapatite make it a widely popular biomaterial for orthopaedic and dental implants [10]. Bone formation in the implanted hydroxyapatite involves complex regulatory roles of soluble mediators and intracellular signaling pathways initiated by the binding between osteoblast-expressed surface integrin molecules and its ligand, *e.g.*, arginine-glycine-aspartic acid (RGD) motif, of the extracellular matrix proteins covering hydroxyapatite surfaces [11]. Previous studies showed that hydroxyapatite stimulates human osteoblast proliferation via cell surface integrin α V and under the regulation of nitric oxide (NO) [12] [13] [14] [15]. Moreover, PGE₂ was produced by osteoblasts cultured on hydroxyapatite [16] [17]. Therefore, the aim of the present study was to determine whether hydroxyapatite-stimulated human osteoblast proliferation may be directly under the regulation of PGE₂.

2. Materials and Methods

Hydroxyapatite (9% porosity) sintered at 1200°C and cut into 2 × 2 × 2 mm³ in

size were sterilized. This implant material was supplied by the School of Material and Mineral Resource Engineering, Universiti Sains Malaysia. All materials were obtained from Sigma (St. Louis, MO, USA), unless otherwise stated. Osteoblasts (HOS cell line) were purchased from ATCC (Rockville, MD, USA) and cultured in a complete medium containing Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum and 1% penicilline-streptomycin [12]. After harvesting and washing, a single cell suspension (1×10^6 cells/ml) was prepared in the above medium. In order to elucidate the role of COXs, indomethacin (a non-specific COX inhibitor), aspirin (a COX-1 inhibitor) and nimesulide (a COX-2 inhibitor) were used and dissolved in DMSO [17]. Aspirin was a gift from PT. Bayer, Jakarta-Indonesia. One million cells were incubated in 1 ml of the culture medium containing various concentrations of the respective COX inhibitors for 30 minutes at room temperature. After washing, two hundred microliters of cell suspension containing 2×10^5 cells/well in the 96-well culture plates were cultured with or without hydroxyapatite and incubated for 3 days at 37°C in a humidified atmosphere and 5% CO_2 [12].

The next study was to determine the effect of exogenous PGE_2 on the cell cultures with or without hydroxyapatite. One million cells were incubated in 1 ml of the culture medium containing 100 μM of nimesulide. After washing, 2×10^5 cells in 200 μL of culture medium were cultured alone or with hydroxyapatite and added with various concentration of PGE_2 . Determining the effect of low and high concentration of PGE_2 on the cells cultured with or without hydroxyapatite, exogenous PGE_2 at concentration of 1, 10, and 100 μM was added into the cell cultures. All cell cultures were incubated for 3 days.

SQ22536, an adenylyl cyclase inhibitor, and dibutyryl cAMP (Db-cAMP), a cAMP analog, used to determine the role of adenylyl cyclase were dissolved in distilled water to obtain 1 mM of solution [15]. Both materials were purchased from Sigma. One million cells were incubated in 1 ml of the culture medium containing 100 μM of SQ22536 for 30 minutes at room temperature [15]. After washing, 200 μL of cell suspension containing 2×10^5 cells/well were cultured with or without hydroxyapatite stimulation as described above. In some wells, the cultures pre-treated with or without SQ22536 were added with 10 μM of PGE_2 and/or 10 μM of Db-cAMP. All cultures were incubated for 3 days.

Further experiment was carried out to determine the role of protein kinase A (PKA). KT5720, a PKA inhibitor, and forskolin, an adenylyl cyclase activator, were dissolved in DMSO, whereas Db-cAMP was diluted in distilled water to obtain 1 mM of a stock solution. All of them were then filter sterilized. One million cells per one milliliter culture medium were incubated with KT5720 (1 μM) and/or forskolin (10 μM) for 2 hours at room temperature. After washing, the cells were cultured with or without hydroxyapatite. PGE_2 10 μM and/or of Db-cAMP (10 μM) were added into some wells of the cultures. Again, the cell cultures were incubated for 3 days.

In order to delineate the role of EP2 and EP4, PF-04418948, an EP2 antagon-

ist, and L-161,982, an EP4 antagonist, were dissolved in DMSO at the concentration of 1 mM and filter sterilized. One million cells were suspended in 1 ml of serum-free medium in the presence of 10 μ M of PF-04418948 or L-161,982 for 2 hours. Two hundred microliters of cell suspension containing 2×10^5 cells were cultured alone or with hydroxyapatite for 3 days as above. In some wells, 10 μ M of PGE₂ were added in the cultures pretreated or untreated with PF-04418948 and L-161,982. All cell cultures were in triplicate.

The cell proliferation was assessed by adding 100 μ l of 20% methanol into the cell suspension for 10 minutes [12]. After further adding 100 μ l of 0.5% crystal violet for 5 minutes, the cells suspension was then washed. Releasing the dye was carried out by adding 100 μ l of 0.5% crystal violet into the cell suspension for 5 minutes. The solution was read by using a spectrophotometer at 540 nm (Bio-tek-Instrument, Inc., Winooski, Vt). The results were represented as absorbance unit, following subtraction of the reading from the medium only. The levels of PGE₂ and intracellular cAMP were assessed from the culture supernatant using EIA-kits (Cayman Chemical Co., Ann Arbor, MI, USA).

The data were statistically analyzed by a one-way analysis of variance followed by Fischer's least square differences (SPSS co., Chicago, USA).

3. Results

3.1. The Role of Cyclooxygenases (COXs)

The role of COX isoforms on osteoblast proliferation stimulated with hydroxyapatite were studied by using COX inhibitors, *i.e.*, indomethacin, a non-specific COX inhibitor, aspirin, a COX-1 inhibitor, and nimesulide, a COX-2 inhibitor. As seen in **Figure 1(a)**, only pre-treatment with indomethacin and nimesulide but not aspirin resulted in partial suppression of hydroxyapatite-stimulated osteoblast proliferation in a dose-dependent fashion as compared with the COX inhibitor-untreated cell proliferation ($P < 0.05$). In contrast, indomethacin and nimesulide but not aspirin did inhibit fully the production of PGE₂ by cells stimulated with hydroxyapatite ($P < 0.05$) (**Figure 1(b)**). Partial suppression of osteoblasts proliferation but profound inhibition of PGE₂ production due to indomethacin or nimesulide could also be observed in the osteoblast cultures without hydroxyapatite stimulation ($P < 0.05$).

3.2. The Effect of Exogenous PGE₂

When nimesulide-pretreated cells were cultured with or without hydroxyapatite and added with various concentration of PGE₂, the results showed that the suppressive effect of nimesulide on the cell proliferation could be abolished by PGE₂ at the concentration of 10 μ M ($P > 0.05$) (**Figure 2**). Furthermore, gradually increased cell proliferation with or without hydroxyapatite was observed after adding PGE₂ at the concentration of 1 and 10 μ M in the cell cultures ($P < 0.05$) (**Figure 3**). In sharp contrast, 100 μ M of PGE₂ added in the cell cultures resulted in significantly decreased cell proliferation as compared with those without

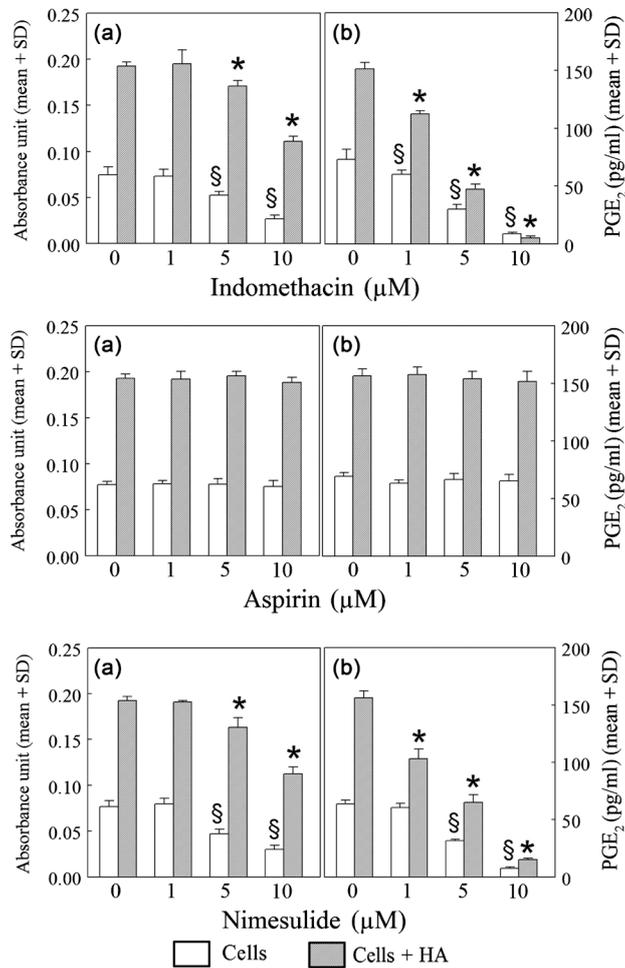


Figure 1. The effect of cyclooxygenase inhibitors on the hydroxyapatite-stimulated osteoblast proliferation (a) and PGE₂ production (b). The cells were pre-treated with indomethacin (non-specific COX inhibitor), aspirin (COX-1 inhibitor) and nimesulide (COX-2 inhibitor) and then cultured with or without hydroxyapatite (HA) for 3 days. (§) significant difference to the cells only (negative control) at P < 0.05. (*) significant difference to the cells + HA (positive control) at P < 0.05.

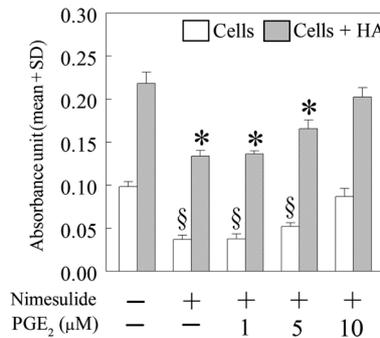


Figure 2. The effect of exogenous PGE₂ on the COX-2 inhibitor-pre-treated and hydroxyapatite-stimulated osteoblast proliferation. The cells were pre-treated with COX-2 inhibitor (nimesulide), and then cultured with or without hydroxyapatite (HA) in the presence or absence of exogenous PGE₂ for 3 days. (§) significant difference to the cells only (negative control) at P < 0.05. (*) significant difference to the cells + HA (positive control) at P < 0.05.

additional PGE₂ ($P < 0.05$).

3.3. The Role of Adenylyl Cyclase

Addition of either PGE₂ or Db-cAMP increased the proliferation of cells cultured with or without hydroxyapatite as compared with that of the untreated cells ($P < 0.05$) (**Figure 4**). Interestingly, the proliferation of SQ22536-pretreated cells cultured with or without hydroxyapatite was significantly lower as compared with that of SQ22536-untreated cells ($P < 0.05$). Suppressed proliferation of SQ22536-pretreated cells cultured with or without hydroxyapatite was abolished by the presence of Db-cAMP ($P > 0.05$) but not PGE₂ ($P < 0.05$). Combination of Db-cAMP and PGE₂ added to the SQ22536-pretreated cells resulted in the cell proliferation comparable with that of SQ22536-untreated cells ($P > 0.05$), suggesting that the action of PGE₂ on the proliferation of cells with or

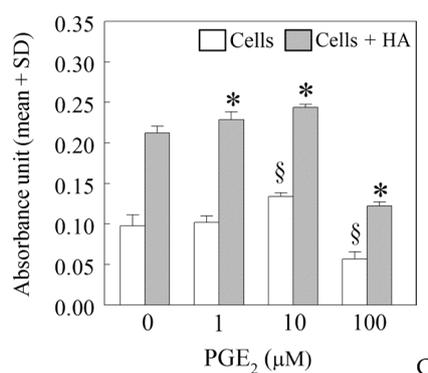


Figure 3. The effect of exogenous PGE₂ on the hydroxyapatite-stimulated osteoblast proliferation. The cells were cultured with or without hydroxyapatite (HA) in the presence or absence of exogenous PGE₂ for 3 days. (§) significant difference to the cells only (negative control) at $P < 0.05$. (*) significant difference to the cells + HA (positive control) at $P < 0.05$.

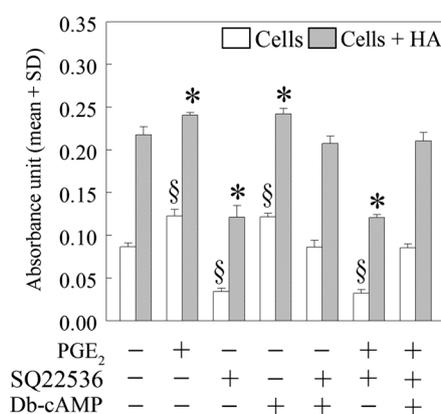


Figure 4. The cAMP-PGE₂ linkage on hydroxyapatite-stimulated osteoblast proliferation. The cells were pre-treated with adenylyl cyclase inhibitor (SQ22536) and then cultured with or without hydroxyapatite (HA) in the presence or absence of cAMP analog (Db-cAMP) and/or PGE₂ for 3 days. (§) significant difference to the cells only (negative control) at $P < 0.05$. (*) significant difference to the cells + HA (positive control) at $P < 0.05$.

without hydroxyapatite may be an adenylyl cyclase-dependent mechanism.

3.4. The Role of Protein Kinase A (PKA)

A PKA inhibitor, KT5720, was used to determine the central role of PKA on the mechanism of PGE₂ on osteoblast proliferation induced by hydroxyapatite. The results showed that additional exogenous PGE₂, Db-cAMP, or forskolin enhanced the proliferation of cells stimulated with or without hydroxyapatite ($P > 0.05$) (Figure 5). In contrast, KT5720 did suppress the cell proliferation stimulated with hydroxyapatite ($P > 0.05$). Suppressed cell proliferation mediated by KT5720 on the cell cultures with or without hydroxyapatite could not be abolished by adding exogenous PGE₂, Db-cAMP or forskolin alone or combination of PGE₂ and Db-cAMP or forskolin ($P > 0.05$). The results indicated that the activation of PKA may prerequisite for the action of PGE₂ on the osteoblast proliferation stimulated with or without hydroxyapatite.

3.5. The Role of EP2 and EP4

The role of EP2 and EP4 on the action of PGE₂ toward the osteoblast proliferation stimulated with or without hydroxyapatite were studied by using their antagonists, *i.e.*, PF-044189448 and L-161,982, respectively. When the cells were pretreated with PF-044189448 or L-161,982 and then cultured with or without hydroxyapatite, the cell proliferation and cAMP production were significantly lower as compared with that treated with or without PGE₂ ($P < 0.05$) (Figure 6(a) and Figure 6(b)). Addition of PGE₂ into EP antagonists-pretreated cells only partially restored the proliferation as well as cAMP production of cell cultured with or without hydroxyapatite ($P < 0.05$). It should be noted, however, that comparing the effects of PF-044189448 and L-161,982 on the cell cultures with or without hydroxyapatite, both cell proliferation and cAMP production

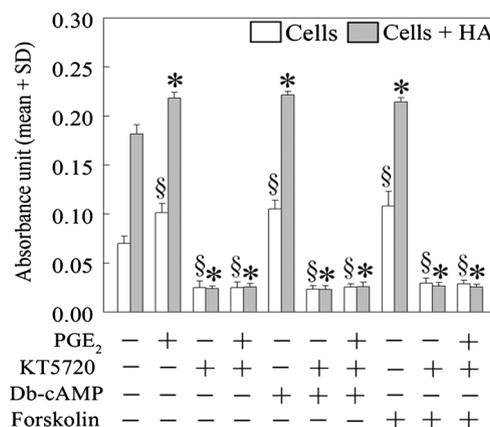


Figure 5. The protein kinase A (PKA)-PGE₂ linkage on hydroxyapatite-stimulated osteoblast proliferation. The cells were pre-treated with PKA inhibitor (KT5720) and then cultured with or without hydroxyapatite (HA) in the presence or absence of cAMP analog (Db-cAMP), adenylyl cyclase activator (forskolin) and/or PGE₂ for 3 days. (\$) significant difference to the cells only (negative control) at $P < 0.05$. (*) significant difference to the cells + HA (positive control) at $P < 0.05$.

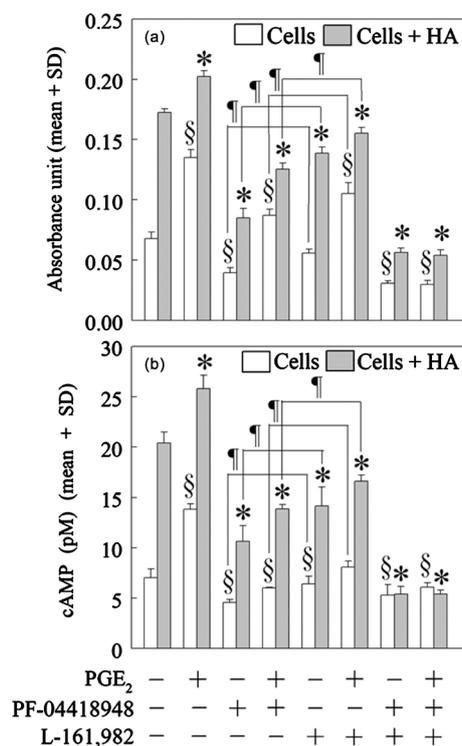


Figure 6. The EP2 and EP4 usage by PGE₂ on hydroxyapatite-stimulated osteoblast proliferation (a) and cAMP levels (b). The cells were pre-treated with EP2 antagonist (PF-04418948) or EP4 antagonist (L-161,982) and then cultured with or without hydroxyapatite (HA) in the presence or absence of PGE₂ for 3 days. (§) significant difference to the cells only (negative control) at $P < 0.05$. (*) significant difference to the cells + HA (positive control) at $P < 0.05$. (¶) significant difference at $P < 0.05$.

suppressed by the former antagonist was much lower than those suppressed by the later one ($P < 0.05$). Adding PGE₂ in PF-044189448 or L-161,982-pretreated cell cultures also resulted in different degrees of the cell proliferation and cAMP production in such that PGE₂ did induce both parameters of PF-044189448-pretreated cell cultures lower than those of L-161,982-pretreated ones with or without hydroxyapatite ($P < 0.05$). Interestingly, the proliferation and cAMP production of cells pre-treated with combined PF-044189448 and L-161,982 and cultured with or without hydroxyapatite were significantly lower than that of cells pretreated with either one of those EP antagonists ($P < 0.05$). Addition of PGE₂ failed to abolish the suppressive effect of combined PF-044189448 and L-161,982 on both cell proliferation and cAMP production of cultures with or without hydroxyapatite ($P < 0.05$) (Figure 6(a) and Figure 6(b)). These results may suggest that the action of PGE₂ on osteoblast proliferation stimulated with or without hydroxyapatite may be via both EP2 and EP4 receptor and that PGE₂-EP2 binding may be predominant over PGE₂-EP4 binding on inducing osteoblast proliferation stimulated with or without hydroxyapatite.

4. Discussion

The present study showed that whilst nimesulide does fully inhibit PGE₂ pro-

duction by osteoblasts after stimulation with or without hydroxyapatite, this COX-2 inhibitor only partially suppresses cell proliferation from the same cultures, suggesting that PGE₂ mediated by COX-2 may partially involve in the osteoblast proliferation induced by hydroxyapatite. Although a previous report revealed COX-2-dependent PGE₂ production by hydroxyapatite-stimulated osteoblast [17], the exact reason(s) by which partial cell proliferation was due to COX-2 inhibition remains unclear. During bone formation, osteoblast differentiation and proliferation require not only PGE₂ but also other growth factors/mediators such as NO and insulin-like growth factor-1 (IGF-1) [18]. Thus, it is conceivable that partial cell proliferation with or without hydroxyapatite as a result of COX-2 inhibition may be due to the regulatory functions of other growth factors/cytokines/mediators on osteoblast proliferation. Indeed, this notion is supported by the current study that the suppressive effect of COX-2 inhibitor on osteoblast proliferation even without hydroxyapatite could be abolished by exogenous PGE₂, indicating that the COX-2-mediated PGE₂ pathway may be a part of the multiple signal transduction networks which regulate osteoblast proliferation and that hydroxyapatite-stimulated PGE₂ may further augment this signaling network (Figure 7).

Intriguingly, exogenous PGE₂ at concentration of 10 μM and 100 μM did promote and suppress the cell proliferation, respectively, suggesting that the effect PGE₂ on osteoblast proliferation with or without hydroxyapatite may be in a dose-dependent fashion. Reports showing that adding low concentration or short time exposure of PGE₂ resulted in increased bone formation [19] [20] may explain the results of the current study. Alternatively, low levels of PGE₂ on osteoblasts might stimulate the production of endothelial nitric oxide synthase (eNOS)-generated NO which might in turn enhance cell proliferation with or without hydroxyapatite [12] [13]. In sharp contrast, high concentration of PGE₂ might lead to osteoblast death, possibly, via PGE₂-activated Wnt antagonist secreted frizzled-related protein-1 (sFRP-1) [20] [21] [22]. Furthermore, these results may have a clinical implication in the dental implantation. Comparing between peri-implant crevicular fluid of healthy and inflamed dental implant sites showed that the levels of fluid PGE₂ from the later implant sites were much higher than those from the former one [9] [23] [24]. It seems plausible, therefore, that whilst low levels of PGE₂ may induce bone formation in the surrounding implanted site and hence, implant stability, high levels of this prostanoid derived from both endogenous and exogenous sources may result in osteoblast death, thereby inducing implant loosening. These notions need to be further determined, however.

That adenylyl cyclase inhibitor-mediated suppression of cell proliferation was abolished by cAMP analog suggests that osteoblast proliferation may be dependent on the activation of cAMP and that hydroxyapatite stimulation on osteoblast proliferation may amplify the cAMP pathway as also previously documented [14] [15]. Interestingly, failure of exogenous PGE₂ to overcome the SQ22536-

suppressed cell proliferation indicates that the role of this prostanoid on osteoblast proliferation with or without hydroxyapatite may also depend on the cAMP activation. Previous findings showing that the stimulatory effect of PGE₂ on the release of osteoblasts-derived IL-1 [25], IL-6 [26] and bone matrix metalloproteinase-1 (MMP-1) [27] is regulated by the cAMP pathway may validate the current study. Furthermore, since hydroxyapatite-induced osteoblast proliferation was also a c-AMP-dependent manner [15], the assumption that PGE₂ action on osteoblast proliferation was to augment the cAMP pathway initially activated by hydroxyapatite should not be ruled out (Figure 7).

Failure of PGE₂ to eliminate the suppressive effect of a PKA inhibitor on the cell proliferation even in the presence of cAMP analog or adenylyl cyclase activator strongly suggests that PKA may play a central role in the PGE₂ action on osteoblast proliferation stimulated with or without hydroxyapatite. A support can be drawn from the fact that the effect of PGE₂ on osteoblasts in releasing cytokines or MMP-1 [25] [26] [27], or regulating its AMP-activated protein kinase (AMPK) [28] are all a PKA-dependent mechanism. Yet again, a possibility that the effect of PGE on osteoblast proliferation induced by hydroxyapatite is also to amplify the same signal transduction pathway is imminent. It should be noted, however, that partial suppression of hydroxyapatite-stimulated osteoblast proliferation by a COX-2 inhibitor was observed in the current study. Therefore, it seems plausible that although COX-2 was inhibited, osteoblast-derived PKA

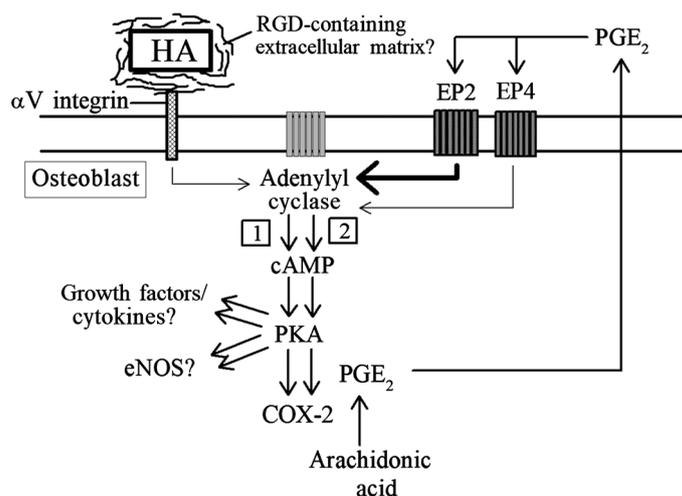


Figure 7. A simplified model of PGE₂ action on hydroxyapatite-stimulated osteoblast proliferation. Following the binding between extracellular matrix-derived RGD motifs covering hydroxyapatite (HA) surfaces [11] and osteoblast-expressed surface α_V integrin molecules [12], adenylyl cyclase and hence, a cAMP pathway is generated (1) thereby leading to PKA activation. Multiple signals encoding the production of cyclooxygenase-2 (COX-2) enzyme and other mediators such as endothelial nitric oxide synthase (eNOS) and growth factors are generated by activated PKA. The action of COX-2 enzyme catalyzes the production PGE₂ from arachidonic acid and acts on the hydroxyapatite-stimulated osteoblasts in an autocrine fashion via both EP2 and EP4, thereby amplifying the existing signals of the cAMP-PKA pathways (2) and hence, up-regulating cell proliferation. Note: a bold arrow represents a dominant signal.

activation initially induced by hydroxyapatite and then augmented by PGE₂ might still be able to generate signal transduction necessary to induce production of other growth factors/cytokines [25] [26] [27] that involve in osteoblast proliferation (Figure 7). This contention is in accordance with wealth of evidences indicating that PKA may act as a crossroad-like site where multiple signals could be generated to activate multiple genes encoding multiple mediators [29]. Further studies are required to validate the above contention.

Out of four receptor subtypes specific for PGE₂, EP2 and EP4 are known to activate the cAMP-PKA pathway [1]. Therefore, the next experiment was to determine whether the role of PGE₂ on hydroxyapatite-stimulated osteoblast proliferation is mediated by EP2 and/or EP4. The results of this study showed that the effect of PGE₂ on osteoblast proliferation without stimulation of hydroxyapatite is via EP2 and EP4. These results are in accordance with a previous work demonstrating the EP2 and EP4 usage by PGE₂ on osteoblasts derived from EP2 or EP4 gene knock out and its relevant wild type mice [2] [3]. Surprisingly, previous reports demonstrating that the stimulatory effect of PGE₂ on hydroxyapatite-induced osteoblast proliferation is mediated via EP2 and EP4 are still lacking. The work of Sanuki and colleagues [30] indicating that application of mechanical forces onto human osteoblasts (Saso-2 cells) results in an increased PGE₂ production and EP2/EP4 expression may support the results of this study. Furthermore, the present study also indicated that the usage of EP2 by PGE₂ was predominant over that of EP4 in osteoblast proliferation with or without hydroxyapatite. It was previously found that EP2 agonists on murine osteoblasts induce cAMP production much higher than EP4 agonists [31] and that EP2 and EP4 act independently due to their differences in generating specific MAPK signaling pathways [32]. Thus, PGE₂-elevated osteoblast proliferation and cAMP production of the cell cultures with or without hydroxyapatite may be due to greater signal transduction generated from activated EP2 than EP4 (Figure 7). However, this contention needs to be investigated further.

5. Conclusion

The PGE₂ production and partial cell proliferation by human osteoblasts stimulated with hydroxyapatite were a COX-2-dependent mechanism. The cell proliferation cultured with hydroxyapatite was enhanced and suppressed by low and high concentration of PGE₂, respectively. The adenylyl cyclase inhibitor-suppressed cell proliferation was abolished by cAMP analog but not exogenous PGE₂. Suppression of hydroxyapatite-induced cell proliferation by a PKA inhibitor could not be eliminated by cAMP analog and/or adenylyl cyclase activator. The COX-2-mediated PGE₂ acted on hydroxyapatite-stimulated osteoblast proliferation via both EP2 and EP4 but the former receptor was more dominant than the latter one. Therefore, the current study suggests that human osteoblast proliferation induced by hydroxyapatite may be partially regulated by PGE₂ acting in an autocrine fashion via a COX-2-, cAMP-PKA- and both EP2 and EP4-dependent

pathway.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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