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Differential effects of prostaglandin E_2 and enamel matrix derivative on the proliferation of human gingival and dermal fibroblasts and gingival keratinocytes

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Background and Objective: Elevated levels of prostaglandins contribute to periodontal destruction but can impair gingival healing by affecting local fibroblasts. Enamel matrix derivative (EMD) has beneficial effects on supporting and gingival tissues. We showed that prostaglandin E_2 (PGE_2) inhibits the proliferation of human gingival fibroblasts (hGFs) and that EMD stimulates it. Prostaglandins and EMD may also affect skin healing by targeting dermal fibroblasts (DFs). Thus, we compared the effects of these two agents on the proliferation of hGFs, human gingival keratinocytes (hGKs) and hDFs.

Material and Methods: Cells from healthy human gingiva or skin were treated with PGE_2 and/or EMD, and proliferation was assessed by measuring cell number and DNA synthesis.

Results: In hGFs, PGE_2 (1 μM) inhibited proliferation while EMD stimulated it. When present together, EMD abolished the PGE_2-induced inhibition. Serum increased (by a factor of 10) the amount of phosphorylated extracellular signal-regulated kinase (p-ERK), PGE_2 reduced it (by 70–80%) and EMD restored it when present with PGE_2. Prostaglandin E_2 stimulated cAMP production in hGFs while serum or EMD did not. Enamel matrix derivative stimulated hDF proliferation, but the inhibitory effect of PGE_2 was milder than with hGFs. When present together, EMD abolished the PGE_2-induced inhibition. Enamel matrix derivative inhibited the proliferation of primary hGKs, but PGE_2 had no effect. Finally, we found that hDFs contained about five times less prostaglandin EP_2 receptor mRNA than hGFs, while hGKs contained none.

Conclusion: Prostaglandin E_2 inhibits and EMD stimulates hGF proliferation via distinct pathways. The different sensitivities of hDFs and hGKs to PGE_2 can be explained by the levels of EP_2 expression.

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Prostaglandins (PGs) such as PGE_2 are pleiotropic molecules and as such affect many physiological processes and participate in many diseases. In periodontal disease, PGs are secreted in large amounts (1,2) and contribute to the inflammatory process as well as mediate the induction of bone resorption (3–5). In acute skin wound healing, PGs also have a physiological role in the initial inflammatory phase (6). However, exaggerated local production of PGs due to trauma or infection of the wound may interfere with proper cutaneous healing. In addition to its recognized role in the inflammation *per se*, PGE_2 has documented antiproliferative effects on gingival (7–10) and dermal fibroblasts (11,12) as well as on fibroblasts from other sources [tendon (13), lung (14), embryonic (15) and liver (16)] and on other cell types, such as gastric carcinoma cells (17) and arterial smooth muscle cells (18). Interestingly, there are no solid data on whether PGE_2 affects the proliferation of gingival epithelial cells (keratinocytes).

Prostaglandin E_2 acts on a variety of cells via cell-surface G-protein-coupled receptors, which are divided into four subtypes, $EP_{1–4}$, that differ in their signal transduction pathways. Of these four receptors, EP_4 and EP_2 activate adenylate cyclase and increase intracellular levels of cAMP, EP_1 activates phospholipase C (PLC) and EP_3 either lowers cAMP levels or activates PLC, depending on the alternatively spliced isoform (19,20). In recent years, EP_2 has emerged as the major receptor mediating the antiproliferative action of PGE_2 in gingival (10), lung (14,21) and liver fibroblasts (16).

Enamel matrix proteins are secreted by ameloblasts during odontogenesis and regulate enamel mineralization (22,23), but are also secreted by epithelial cells during root formation and affect cementogenesis and the formation of the periodontal supporting tissues (24,25). As a result of these observations, Emdogain® [enamel matrix derivative (EMD, Institut Straumann, Basel, Switzerland)] is being successfully applied for periodontal regenerative surgical treatment (26–28). In addition to affecting periodontal ligament and osteoblastic cells *in vitro*, we have published extensive data on the beneficial (mitogenic and antiapoptotic) effects of EMD on human gingival fibroblasts (29–32). In addition, there is very little evidence that EMD may stimulate the proliferation of dermal fibroblasts (although using complex *in vitro* models; 33,34) and may also inhibit the proliferation of gingival epithelial carcinoma cell lines (35–37). Information on the effects of EMD on the proliferation of primary dermal fibroblasts in monolayer cultures and normal gingival keratinocytes is lacking.

Emdogain is applied clinically for treatment of periodontal loci afflicted with inflammation and may consequently coexist with inflammatory mediators such as PGE_2 at the treated site, a fact that may potentially interfere with its actions. In addition, the emerging interest in applying Emdogain for treatment of skin wounds (28) and the potential situation where EMD will be placed onto an inflamed skin led us to test its activity on the proliferation of dermal cells either alone or in the presence of PGE_2. Thus, the purpose of this study was to use primary human nontransformed gingival fibroblasts, dermal fibroblasts and gingival keratinocytes in order to test the potentially contrasting effects of PGE_2 and EMD on their proliferation.

Material and methods

Materials

All reagents for fibroblast cultures were from Biological Industries (Beit Haemek, Israel). Tissue culture dishes were from Nunc (Roskilde, Denmark), crystal violet was from Edward Gurr (London, UK) and trichloracetic acid (TCA) from Sigma (St Louis, MO, USA). [^{3}H]Thymidine was from Perkin Elmer (Boston, MA, USA). Prostoglandin E_2 was from Biomol (Plymouth Meeting, PA, USA) and was dissolved in 100% ethanol at 10^{-2} M and further diluted in culture medium, so that the final ethanol concentration was always less than 0.1%. Keratinocyte culture medium (KGM® CD BulletKit) was from Lonza (Basel, Switzerland). Enamel matrix derivative (EMD) was generously donated by Institut Straumann AG (Basel, Switzerland).

Primary antibodies to phospho-ERK and ERK (both recognizing ERK-1 and ERK-2) were from Sigma. Peroxidase-conjugated antimouse or antirabbit IgG, respectively, were from Jackson (West Grove, PA, USA).

Fibroblast isolation and culture

The experiments were approved by the Helsinki committee of the Tel-Aviv University and Hillel Yaffe Medical Center. Informed consent was obtained from all patients. Gingival fragments from periodontal or implant procedures were separated into connective tissue and epithelium using dispase (Gibco, Grand Island, NY, USA) at 2 mg/mL for 2 h at 37°C, and the connective tissue was cut into fragments, which were placed in culture medium [α-minimal essential medium supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 12.5 U/mL nystatin, 0.11 mg/mL sodium pyruvate and nonessential amino acids] at 37°C in a humidified atmosphere of 5% CO_2 and 95% air to allow for cell outgrowth. The medium was replaced every 3 d until confluence was reached. Cells at the second or third passage, having a typical fibroblastic morphology, were used. Any well with epithelial contamination was discarded. Dermis fragments from cosmetic eyelid or breast surgery were treated like gingival connective tissue.

Gingival keratinocyte culture

Gingival epithelial tissue was dissociated into single cells with crystalline trypsin (Biological Industries; 0.02% for 5 min at 37°C with agitation), which was then neutralized by soybean trypsin inhibitor (Biological Industries). Keratinocytes were seeded in 96-well plates coated with fibronectin (5 μg/mL), and medium [KGM-CD Keratinocyte Growth Medium including its supplements (CC-4456), Lonza] was replaced every 2 d.

Cell number

Cell number was determined colorimetrically using crystal violet as previously described (29). Fibroblasts were plated at 50,000 cells per well in 24-well plates in triplicate, and allowed to attach for 24 h in a medium containing 10% FCS. Cells were then starved for 24 h in a serum-free medium and then challenged with different medium combinations (5% FCS as proliferation stimulant + PGE_2 or EMD at various concentrations), and cell number was determined 48 h later. Keratinocytes were plated at 1000 cells per well in 96-well plates in quintuplicate and allowed to attach for 24 h in keratinocyte culture medium. Fresh medium was then added, containing PGE_2 or EMD at various concentrations, and cell number was determined every 24 h, up to 5 d in culture.

Thymidine incorporation

Thymidine incorporation was assayed as described previously (29). Cells were seeded at 50,000 cells per well in 24-well plates in triplicate, allowed to spread and starved as described above. Cells were then challenged with different medium combinations (5% FCS as proliferation stimulant + PGE_2 or EMD at various concentrations), and 20 h later [^{3}H]thymidine was added at a final concentration of 1 μCi/mL for 4 h, and cells were washed three times with phosphate-buffered saline. The DNA was precipitated with 5% TCA for 45 min on ice, solubilized with 0.5 N NaOH for 90 min at room temperature, and the radioactivity in the cell lysate was determined in a Beckman® LS-6000SC Liquid Scintillation Counter.

Measurements of cAMP

Intracellular cAMP measurements were performed with the cAMP Biotrak EIA kit from Amersham (Little Chalfont, UK). Cells (50,000 per well) were incubated with 5% FCS with or without PGE_2 or EMD for 15 min, followed by lysis and measurements according to the manufacturer's instructions.

Western blot analysis

Cells (500,000 per 60 mm culture dish) were treated with 5% FCS ± PGE_2 ± EMD for 10–16 h and then washed with ice-cold phosphate-buffered saline, lysed with sodium dodecyl sulfate (SDS)-sample buffer and boiled for 15 min. Samples were subjected to SDS-polyacrylamide gel electrophoresis in reducing conditions using 10% polyacrylamide gels (20 μg protein per lane) on a TransBlot SD device (Bio-Rad, Hercules, CA, USA). Proteins were transferred to nitrocellulose membranes, which were blocked with TBS (Tris-buffered saline) with 4% (w/v) nonfat dry milk and were probed overnight at 4°C with specific primary antibodies (see below). Membranes were washed with TBS with 0.25% Tween-20 (TBS/T) and 4% (w/v) nonfat dry milk, and bound antibodies were visualized using horseradish peroxidase-conjugated secondary antibodies (1 h at room temperature) and enhanced chemiluminescence reagents (Pierce, Rockford, IL, USA) and Bio-Max light film (Kodak, Rochester, NY, USA). Antibodies (diluted in a buffer composed of 1/8 Wash Buffer and 7/8 TBS/T) were used as follows: anti-phospho-ERK at 1:10,000 with 1:10,000 of the secondary antibody (antimouse); and anti-total-ERK at 1:40,000 with 1:10,000 of the secondary antibody (anti-rabbit).

Isolation of RNA and determination of mRNA by real-time PCR

Total RNA was isolated from 3×10^6 cells per donor (three or four donors per cell type) with the Perfect Pure RNA Cultured Cell Kit (5Prime, Gaithersburg, MD, USA) according to the manufacturer's instructions. The quality and quantity of the RNA was estimated with a nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Only samples with an OD_{260}/OD_{280} ratio > 2 were used. Total RNA (0.5 μg) was then reverse transcribed by EZ-First Strand

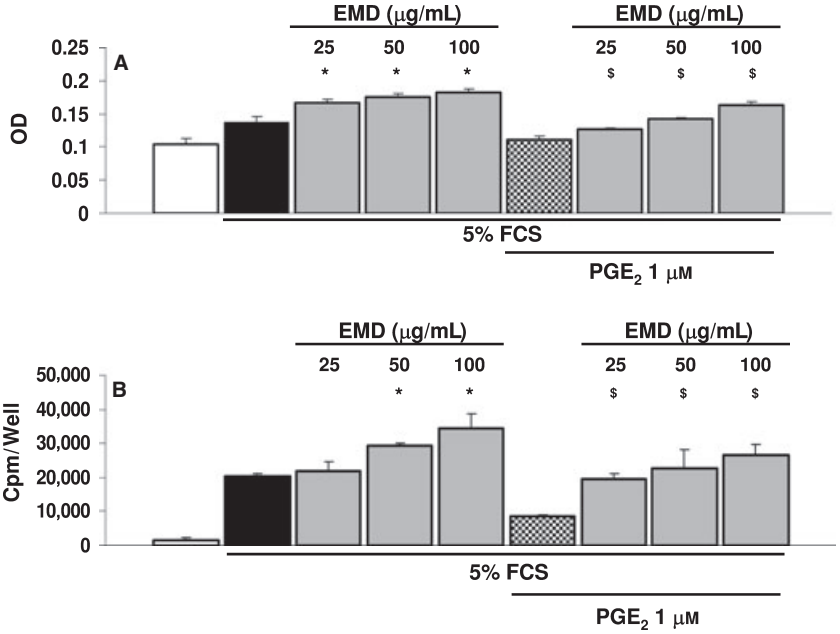

Fig. 1. Prostaglandin E_2 at 1 μM inhibits serum-stimulated proliferation of hGFs while EMD stimulates it in a dose-dependent manner. (A) Cell number is measured colorimetrically by crystal violet staining and presented as optical density (OD) 48 h after stimulation with fetal calf serum (FCS) ± PGE_2 ± EMD at various concentrations. (B) DNA synthesis is measured as thymidine incorporation 24 h after stimulation with FCS ± PGE_2 ± EMD at various concentrations. Open bars represent cells kept in serum-free medium. *$p < 0.05$ vs. control (FCS without PGE_2 or EMD, black-filled bars). \$$p < 0.05$ vs. PGE_2 alone (checkered bars). $n = 3$ or 4 wells per condition.

cDNA Synthesis Kit (Biological Industries) using random hexamer primers according to the manufacturer's instructions. Transcribed cDNA was amplified (denaturation for 10 min at 95°C, 40 cycles of 15 s denaturation (95°C) and 60 s annealing and synthesis at 60°C) using Assay-on-Demand kits (Hs00168754_m1 for EP_2 and Hs99999905_m1 for the endogenous control, *GAPDH*, all from Applied Biosystems, Foster City, CA, USA (http://www3.appliedbiosystems.com/AB_Home/index.htm) and TaqMan Universal PCR Master Mix according to the manufacturer's instructions using a Prism 7000 Thermocycler (Applied Biosystems). Calculations of relative gene expression (normalized to *GAPDH* reference gene) were performed according to the $2^{-\Delta\Delta C_t}$ Method (38).

Statistical analysis

All assays were performed in triplicate/quadruplicate for each condition, and each experiment was repeated at least twice. The results are presented as means and standard deviations (SD). Statistical analysis was performed by analysis of variance followed by *post hoc* multiple comparisons using the least significant difference (LSD) test.

Results

Prostaglandin E_2 at 1 μM inhibited the serum-stimulated proliferation of human gingival fibroblasts (hGFs), measured both as cell number and DNA synthesis, while EMD increased it in a dose-dependent manner (Fig. 1). Furthermore, EMD stimulated hGF proliferation in the presence of PGE_2, thereby protecting the cells from the antiproliferative effect of PGE_2. Serum markedly (~10-fold) increased the amount of phospho-ERK in hGFs, and PGE_2 inhibited it greatly (by 70–80%). Treating the cells with EMD in the presence of PGE_2 restored the amount of phospho-ERK back to control levels (serum alone; Fig. 2), attesting to the important role of activation of the ERK cascade in controlling hGF proliferation. Furthermore,

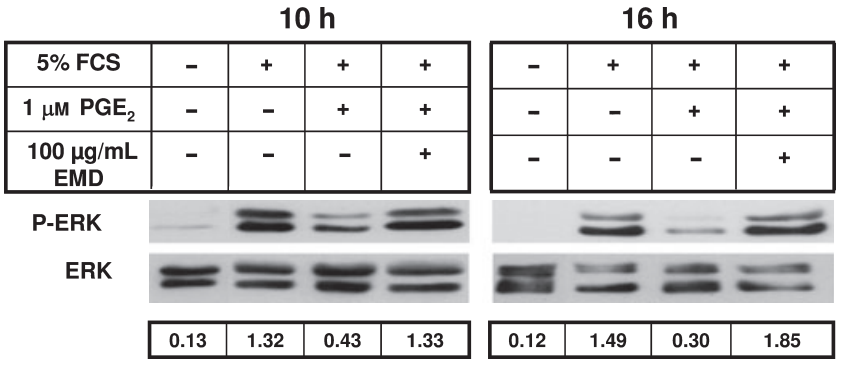

Fig. 2. Representative western blotting analysis of the amount of phospho-ERK (p-ERK) and total ERK (ERK) in hGFs. Cells were incubated with serum-free medium or medium containing 5% FCS or 5% FCS + 1 μM PGE_2 or 5% FCS + 1 μM PGE_2 + EMD at 100 μg/mL for 10 or 16 h. The ratio of p-ERK/ERK derived by densitometry is provided under each lane.

while serum and EMD did not increase the amount of cAMP in hGFs, PGE_2 dramatically stimulated cAMP production (which was not altered by addition of EMD; Fig. 3).

Enamel matrix derivative also stimulated the proliferation of human dermal fibroblasts (hDFs) derived from either eyelid or torso region in a dose-dependent manner (Fig. 4), similar to its effect on hGFs. Prostaglandin E_2, in contrast, inhibited hDF proliferation in a dose-dependent fashion; however, its effect was evidently smaller than with hGFs (Fig. 5, compare A,C with B,D). As in the case of hGFs, EMD, in the presence of PGE_2, gradually restored hDF proliferation to its control (serum alone) rate (Fig. 6). Thus, human dermal fibroblasts are as responsive to the mitogenic effect of EMD as human gingival fibroblasts, but are much less susceptible to the antiproliferative effect of PGE_2.

In agreement with previous reports using an oral carcinoma-derived epithelial cell line (35,36), EMD inhibited the increase in the number of primary human gingival keratinocytes (hGKs) in culture in a dose-dependent manner (Fig. 7). Interestingly, PGE_2 at 1 μM had no effect on hGK cell number.

Finally, in an attempt to find an explanation for why the antimitogenic effect of PGE_2 is weaker in hDFs and absent in hGKs, we measured the amount of the mRNA of the EP_2 receptor, which is emerging as the main receptor mediating the antiproliferative

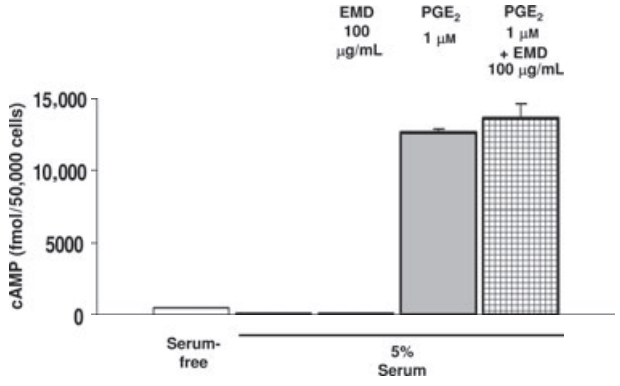

Fig. 3. Neither FCS (5%) nor EMD (100 μg/mL) induce cAMP formation in hGFs. In contrast, PGE_2 at 1 μM causes dramatic cAMP production, which is not affected by concomitant EMD treatment. $n = 3$ or 4 wells per condition.

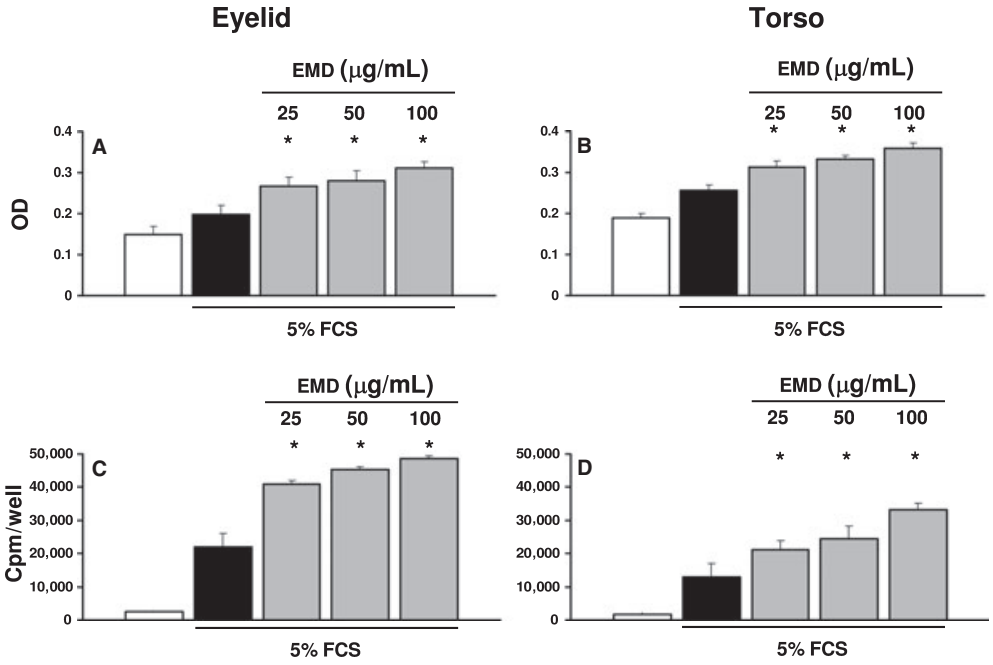

Fig. 4. Enamel matrix derivative augments the proliferation of serum-stimulated human dermal fibroblasts (hDFs) taken from either eyelid (A,C) or torso regions (B,D). Data are given either as optical density (OD) of cell number measured using crystal violet staining after 48 h (A,B) or c.p.m./well of thymidine incorporation at 24 h (C,D). *$p < 0.05$ vs. control (FCS alone, black-filled bars). Open bars represent cells kept in serum-free medium. $n = 3$ or 4 wells per condition.

effect of PGE_2 in many cell types, in hDFs (derived from three donors) in comparison with hGFs (derived from four donors), using quantitative real-time PCR. Our results show that hDFs contain approximately five times less EP_2 mRNA than hGFs, while none could be detected in hGKs (Fig. 8).

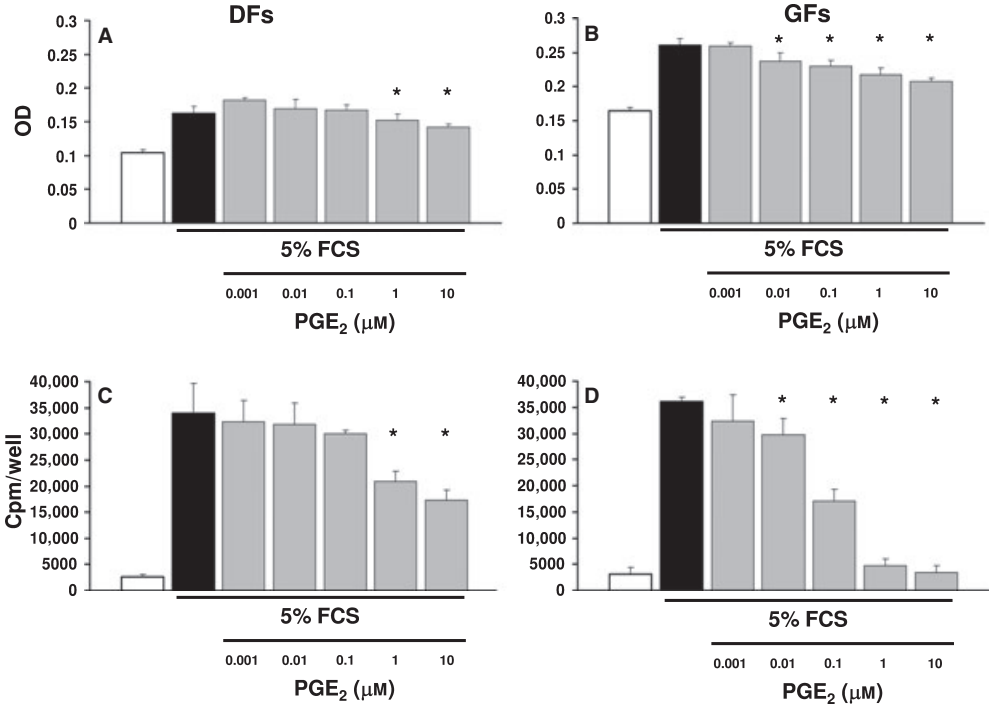

Fig. 5. Prostaglandin E_2 inhibits the serum-stimulated proliferation (measured as cell number at 48 h in A and B and thymidine incorporation at 24 h in C and D) of both hDFs (A,C) and hGFs (B,D) in a dose-dependent manner, but the effect on hGFs is much more pronounced. *$p < 0.05$ vs. control (FCS alone, black-filled bars). Open bars represent cells kept in serum-free medium. $n = 3$ or 4 wells per condition.

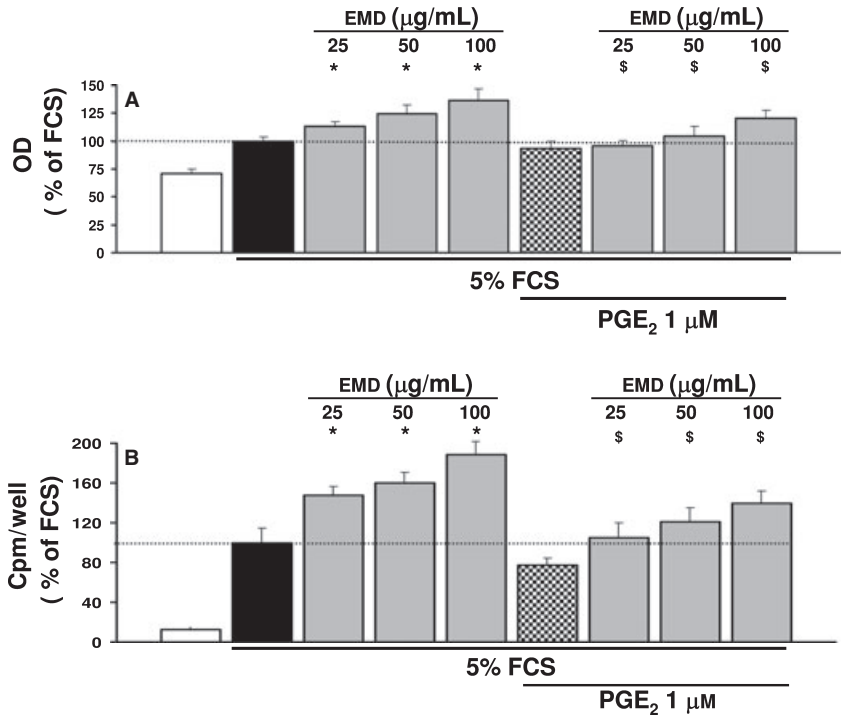

Fig. 6. Prostaglandin E_2 at 1 μM inhibits serum-stimulated proliferation of hDFs while EMD stimulates it in a dose-dependent manner. (A) Cell number is measured colorimetrically by crystal violet staining and presented as optical density (OD) 48 h after stimulation with fetal calf serum (FCS) ± PGE_2 ± EMD at various concentrations. Data are means ± SD of two experiments from two donors each, and the OD of cells treated with 5% FCS is set to 100%. (B) DNA synthesis is measured as thymidine incorporation 24 h after stimulation with FCS ± PGE_2 ± EMD at various concentrations. Data are means ± SD of two experiments from two donors each and the c.p.m./well of cells treated with 5% FCS is set to 100%. $*p < 0.05$ vs. control (FCS without PGE_2 or EMD, black-filled bars). $\$p < 0.05$ vs. PGE_2 alone (checkered bars). Open bars represent cells kept in serum-free medium. $n = 3$ or 4 wells per condition.

Discussion

In agreement with our recent studies (10,29), respectively, PGE_2 inhibited and EMD stimulated hGF proliferation. We report here that when combined together, EMD restored hGF proliferation and abolished the PGE_2-induced inhibition, indicating that EMD can stimulate hGF proliferation in the presence of inflammation-associated molecules such as PGE_2. If extrapolated to the *in vivo* environment, this would suggest that the clinically used EMD preparation (Emdogain®) may beneficially affect gingival connective tissue even in the presence of some degree of local inflammation. Also, in agreement with our studies (10), PGE_2 dramatically inhibited ERK phosphorylation and EMD restored it to the (serum-stimulated) control values. Our past studies and present data indicate that PGE_2 and EMD exert their opposite effects on hGF proliferation via discrete pathways. While PGE_2 inhibits hGF proliferation via the EP_2–cAMP–Epac (exchange protein directly activated by cAMP) pathway (10), EMD stimulates it via cAMP-independent pathways that involve the activity of src kinases and cell-surface matrix metalloproteases, resulting in signaling through the epidermal growth factor receptor (31). These opposing, mechanistically different effects converge at the level of phospho-ERK (present study). This observation, combined with the fact that a MAPK inhibitor (U0126), which prevents ERK phosphorylation, abrogates the mitogenic effect of EMD in GFs (29), points to the crucial importance of activation of the ERK cascade in controlling hGF proliferation. While we could not find any evidence that cAMP production is elicited in human GFs by EMD (in agreement with other studies and reference 36), this was found in other cell types (39,40).

Prostanoids like PGE_2 are found in increased amounts in inflamed gingiva and contribute both to the inflammatory process and to the induction of bone resorption (reviewed in 3, 41–43). By inhibiting the proliferation of gingival fibroblasts, PGE_2 hampers the repair of the gingival connective tissue, which follows the inflammation-associated tissue destruction and cell death. In this respect, PGE_2 collaborates with tumor necrosis factor-α (TNFα), another cytokine strongly associated with the periodontal inflammation, which induces apoptosis of gingival (30) and dermal (44) fibroblasts. Therefore, both TNFα and PGE_2 contribute to the resulting gingival damage by targeting its major cellular component, gingival fibroblasts. In contrast, EMD, which protects GFs from the antimitogenic effect of PGE_2 (this study) and from TNFα-induced apoptosis (30), possesses the ability to counteract the deleterious effects of inflammatory agents and promote gingival healing *in vivo* (45,46).

Our present data indicate that human dermal fibroblasts are as sensitive as gingival fibroblasts to the mitogenic effect of EMD. The only pre-existing data showed that EMD increases DF cell number when these cells were assayed in either in a 'dermal equivalent' construct (33) or in an '*in vitro* wound healing model' (34), both of which involve other cellular inputs (from the surrounding collagen matrix) and activities (migration). Thus, our data indicate that EMD is clearly mitogenic to DFs in the absence of these confounding variables. Future experiments will show whether the mechanisms involved in EMD-induced GF mitogenesis also operate on DFs. Nevertheless, such observations naturally open the road for experiments that will test the ability of EMD to improve skin wound healing *in vivo* (28,47). Notably, EMD stimulates DF

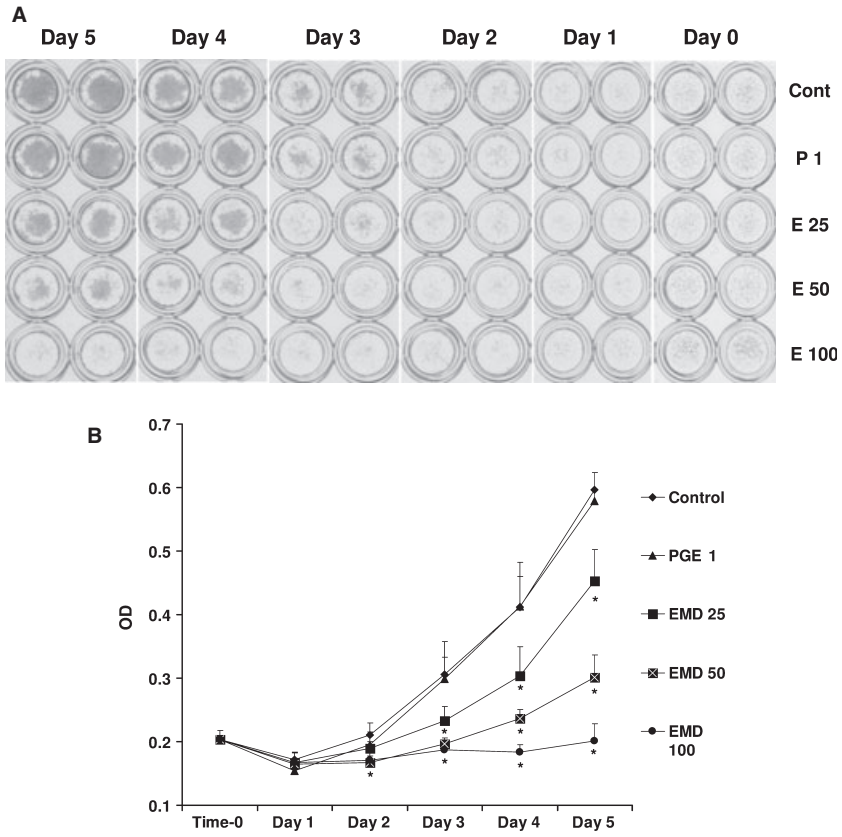

Fig. 7. (A) Crystal violet-stained wells containing hGKs up to 5 d in culture, showing a significant increase in cell number with time in control cultures that is prevented by EMD but not PGE_2. Abbreviations: P 1, PGE_2 at 1 μM; and E 25–E 100, EMD at 25–100 μg/mL. (B) Quantification of staining intensity shows that EMD inhibits hGK proliferation in a dose-dependent manner, while PGE_2 at 1 μM has no effect. $*p < 0.05$ vs. control cells. $n = 4$ wells per condition.

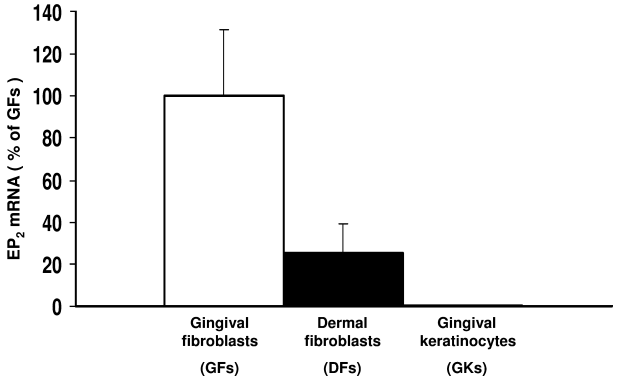

Fig. 8. Quantification by real-time PCR of the amount of EP_2 mRNA in human gingival and dermal fibroblasts as well as gingival keratinocytes, relative to the amount of *GAPDH* mRNA. The amount of EP_2 mRNA in the gingival fibroblasts is set to 100%. $n = 2$–4 donors per cell type, and data are presented as means ± SEM.

proliferation even in the presence of PGE_2, suggesting that it may increase dermal cellular mass even in the presence of inflammatory agents.

In contrast, we found that dermal fibroblasts are less sensitive to the antimitogenic effect of PGE_2 than gingival fibroblasts. Prostaglandin E_2 inhibits proliferation of many cell types, including fibroblasts from various sources (7–18), and in several of these cells it was shown to operate via the EP_2 receptor and cAMP generation (10,12,14,16–18). Like GFs (48), DFs express all four EP receptors (49) and, since PGE_2 causes cAMP production in DFs (50), it is reasonably safe to assume that EP_2 also mediates the inhibitory effect of PGE_2 on DF proliferation. In support of this possibility, we demonstrate here that the amount of EP_2 mRNA in DFs is approximately 5 times lower than that in GFs, and these data could explain why DFs are less susceptible than GFs to the PGE_2-induced inhibition of proliferation.

Prostaglandin E_2 participates in the initial inflammatory phase of acute skin wound healing, which has important functions, such as vasodilatation and increased vascular permeability, to facilitate the ingress of inflammatory cells into the wound area for the removal of debris and bacteria. However, chronic, nonhealing skin wounds, such as burns, infected or traumatized wounds, feature a prolonged and sustained inflammation. In chronic wounds, neutrophils are present throughout the healing period, and the amounts of degradative enzymes (e.g. matrix metalloproteinases) and inflammatory cytokines (e.g. TNFα) are significantly elevated (reviewed in 51, 52). In addition, the concentration of factors that promote fibroblast proliferation and matrix deposition (e.g. platelet-derived growth factor, Transforming growth factor beta) is reduced. Within this environment, high levels of prostaglandins for extended periods may also hamper proper healing, since PGE_2 not only inhibits the proliferation of DFs, but also reduces their migration and collagen contraction (53,54) as well as their production of fibronectin and type I and III collagen (55,56). Interestingly, some of these activities of PGE_2 on fibroblastic cells are also mediated by the EP_2 receptor (53).

There is no substantial evidence that modulating the local production of PGE_2 can affect the proliferation of epidermal or gingival keratinocytes (57,58). Indeed, we found that PGE_2 at

1 μM, a concentration that significantly affects GFs and DFs, does not affect the proliferation of primary gingival epithelial cells, as suggested by Jeng *et al*. (59). Within the gingival environment, epithelial cells are the first barrier against microorganism invasion of the periodontium. As such, they respond to bacterial adhesion and/or bacterial factors such as LPS and initiate the inflammatory process by secreting proinflammatory cytokines (e.g. interleukin-1β, TNFα and interleukin-6) as well as antibacterial molecules such as β-defensins (3). Interestingly, gingival epithelial cells also secrete PGE_2 in response to periopathogens such as *Porphyromonas gingivalis* (60) and *Actinobacillus actinomycetemcomitans* (61); however, this does not seem to affect their own proliferative potential.

In contrast, we clearly show here that EMD inhibits the proliferation of primary normal human gingival keratinocytes. Previously, EMD was shown to inhibit the proliferation of non-oral epithelial (HeLa) cells (39) or oral squamous cell carcinoma-derived cells (35). This is the first report to confirm this effect in nontransformed human gingival keratinocytes. Application of EMD (in the form of Emdogain®) onto the root surface of teeth during periodontal surgical treatment is performed in order to induce the regeneration of cementum, periodontal ligament and alveolar bone. Any proliferation and subsequent apical migration of the gingival epithelial cells may compromise this process. Therefore, the inhibition of epithelial proliferation by EMD can serve this goal by favoring the growth, and later differentiation, of mesenchymal cells (from which the periodontal tissues are derived) within the defect area. Indeed, there are several animal studies suggesting that application of Emdogain® *in vivo* results in shorter postoperative junctional epithelium (62,63).

Conclusion

Enamel matrix derivative, like serum, stimulates hGF proliferation via cAMP-independent pathways, while PGE_2 inhibits it in a cAMP-dependent manner involving the EP_2 receptor. These opposing, mechanistically different effects converge at the level of ERK phosphorylation (which is induced by serum and EMD and inhibited by PGE_2). Human dermal fibroblasts are as sensitive as hGFs to the mitogenic effect of EMD, but are less sensitive than hGFs to the antiproliferative effect of PGE_2, probably due to a much lower expression of the EP_2 receptor. The proliferation of hGKs is not affected by PGE_2, probably due to absence of EP_2 expression; however, it is significantly inhibited by EMD. These differential effects of PGE_2 and EMD may have clinical relevance in periodontology or dermatology in that EMD may stimulate the proliferation of GFs and DFs even in the presence of inflammatory mediators such as PGE_2.

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