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In vitro models of periodontal cells: a comparative study of long-term gingival, periodontal ligament and alveolar bone cell cultures in the presence of β-glycerophosphate and dexamethasone

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Abstract Human gingival (HG), periodontal ligament (HPL) and alveolar bone (HAB) cells (first subculture) were cultured (10^4 cells/cm^2) for 35 days in α-Minimal Essential Medium supplemented with 10% fetal bovine serum in the presence of (i) ascorbic acid (AA, 50 μg/mL), (ii) AA + β-glycerophosphate (βGP, 10 mM) and (iii) AA + βGP + dexamethasone (Dex, 10 nM). Cultures were assessed for cell attachment and spreading, cell proliferation, alkaline phosphatase (ALP) and acid phosphatase (ACP) activities and matrix mineralization. HG cell cultures presented a high proliferation rate, a low ability to synthesize ALP and ACP and the formation of a non-mineralized extracellular matrix, regardless the experimental situation. HPL cell cultures were very sensitive to the culture conditions and showed a high proliferation rate, synthesis of moderate levels of ALP and ACP and a modest matrix mineralization in the presence of AA + βGP + Dex. HAB cell cultures presented a growth rate lower than that of HG and HPL cells, a high ALP activity and comparatively low levels of ACP, and the ready formation of a heavy mineralized matrix in the presence of βGP. In the three periodontal cell cultures, Dex enhanced cell proliferation and expression of osteoblastic markers. Results showed that βGP and Dex allowed the modulation of the cell proliferation/differentiation behavior within the proposed physiological and regenerative capabilities of these periodontal cells.

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Introduction

The periodontium, the supporting teeth apparatus, consists of four types of connective tissues, two fibrous tissues, the gingival lamina propria and the periodontal ligament, and the mineralized cementum and alveolar bone [1].

Gingiva is the portion of the oral mucosa that covers the tooth-bearing part of the alveolar bone and the cervical neck of the tooth and the periodontal ligament is the dense connective tissue located between the alveolar bone and the root surface. Fibroblasts are the predominant cell type residing in these soft connective tissues and have been shown to differ in terms of size, proliferative ability and expression of extracellular molecules [2–9]. Also, evidence exists that the periodontal ligament expresses a mineralized tissue-forming cell lineage which comprises the osteoblastic and the cementoblastic phenotypes [1, 10–12]. In addition, differences have been reported within each of the gingival and periodontal ligament fibroblast population [1, 13–15]. Cementum is a mineralized tissue usually limited to the surface of the tooth root and shares

many properties with the bone tissue, but is avascular, non-innervated and possesses low remodeling potential [1]. By contrast, alveolar bone, the part of the maxilla or mandible which supports and protects the teeth, has a very high turnover rate. Like the fibroblasts, osteoblasts, the bone-forming cells, originate from mesenchymal stem cells and through a series of commitment and differentiation steps acquire ability to synthesize the extracellular matrix of bone and regulate its mineralization [1]. Several studies suggest that the osteoblastic phenotype is characterized by extensive plasticity and marked heterogeneity exists in both protein and mRNA expression levels of various osteoblast related markers [16].

Periodontal regeneration involves the structural and functional rebuilding of the tooth's supporting tissues lost as a result of periodontal disease [1, 17, 18]. The diverse composition of the periodontium makes periodontal wound healing a complex process because of the interaction between hard and soft connective tissues, implying the selective repopulation of the root surface by cells capable of reforming the cellular and extracellular components of new periodontal ligament, cementum and alveolar bone [1, 17, 18]. A long list of biomaterials and biological agents have been used with varying success in periodontal regeneration procedures, namely to accomplish the reconstruction of lost attachment apparatus in deep intraosseous defects [18, 19]. General conclusions about the expected clinical benefit or treatment related adverse effects of the different procedures are limited by the marked variability in results due to the lack of objective standardized methodologies [19]. Furthermore, a detailed understanding of the cellular responses to the biomaterials/biologicals procedures is complicated by the diversity of cells and tissues of the periodontium. In this context, human in vitro models representative of the periodontal tissues are useful tools to perform research on periodontal cells/biomaterials, biologicals interactions.

Expression of a particular phenotype in culture depends on the biological material and its manipulation and the culture conditions, namely the culture medium, the culture time and the presence of compounds that influence cell proliferation and differentiation [20–23]. Therefore, the selection of suitable experimental conditions is essential in order to obtain cell cultures with a defined and reproducible behavior. This is particularly important in tissues like the periodontal ligament that can express different phenotypic features. The aim of the present work was to compare the long-term behavior of human gingival (HG), periodontal ligament (HPL) and alveolar bone (HAB) cells cultured in the presence of ascorbic acid (AA) β-glycerophosphate (βGP) and dexametanose (Dex). These medium supplements are frequently used in the culture of connective tissues, including the periodontal cells. However, the diversity of in vitro methodologies reported and the frequent use of animal cells make it difficult to have well-defined models and therefore to draw consensus conclusions in similar studies. Furthermore, to the best of our knowledge, a systematic comparative study regarding the differential effects of these supplements in the long-term proliferation/differentiation behavior of these periodontal cells has not been reported. In the present work, periodontal cells were cultured in the presence of AA, AA + β-βGP and AA + βGP + Dex and cell behavior was assessed in terms of attachment and spreading, proliferation, alkaline phosphatase (ALP) and acid phosphatase (ACP) activities and potential for osteogenic differentiation.

Materials and methods

Culture of human periodontal cells

Primary cultures were obtained by culturing explants of the periodontal tissues from three patients (aged 20–40-years old) undergoing premolar and third molar extractions for orthodontic reasons. Informed consent to use these biological tissues, that would be otherwise discarded, was obtained. In each experiment, cultures of HG, HPL and HAB cells were established from the same patient. The periodontal tissues were cut into small pieces, cultured in separate cell-culture plates in α-Minimal Essential Medium supplemented with 10% fetal bovine serum, 50 μg/mL gentamicin and 2.5 μg/mL fungizone. Cell outgrowth from the tissue explants was observed 1–2 weeks after the beginning of the incubation. HG and HPL cell cultures showed a high proliferation rate and reached confluency approximately 1–2 weeks after the observation of the first cells. HAB cells presented a slower proliferation rate and confluency was attained later, about a week after HG and HPL cell cultures. Primary cultures were passaged (trypsin-EDTA solution) after reaching 70–80% confluency.

First passage HG, HPL and HAB cells were seeded, in separate, at 10^4 cells/cm^2, and cultured for 35 days in the presence of (i) 50 μg/mL AA, (ii) 50 μg/mL AA + 10 mM βGP and (iii) 50 μg/mL AA + 10 mM βGP + 10 nM Dex. This culture period was used based in previous data regarding the time required for the complete expression of the osteoblastic phenotype in

HAB cell cultures [24]. Cultures were assessed for cell attachment and spreading, at 30 min, 1, 2, 6 and 24 h, and for cell proliferation, ALP and ACP activities and matrix mineralization, at days 3, 7, 14, 21, 28 and 35.

Cultures were incubated in a humidified atmosphere of a 5% CO_2 in air at 37 °C and the medium was changed twice a week

Cell viability/proliferation and total protein content

MTT assay—reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a purple formazan product by viable cells—was used to estimate cell viability/proliferation. Cells were incubated with 0.5 mg/mL of MTT for the last 4 h of the culture period tested; the medium was then decanted, formazan salts were dissolved with dimethylsulphoxide and the absorbance (A) was determined at $\lambda = 600$ nm in a microplate reader spectrometer. Results are expressed as A/cm^2. Protein content was determined in 0.1 M NaOH cell lysates according to the method of Lowry using bovine serum albumin as a standard.

ALP and ACP activities

Activities of ALP and ACP were determined in cell lysates (obtained by treatment of the cell layers with 0.1% triton) and assayed by the hydrolysis of *p*-nitrophenyl phosphate, respectively, in alkaline buffer solution (pH 10.3) and citrate buffer solution (pH 4.8). Hydrolysis was carried out for 30 min at 37 °C and the *p*-nitrophenol formed was measured at $\lambda = 405$ nm. Results are expressed as nanomoles of *p*-nitrophenol produced per min per μg of protein (nmol/min μg protein).

Quantification of ionized calcium in the culture medium

Culture media from cell cultures were collected at each change medium and were analysed for ionized calcium (Cai) concentration using a calcium kit (Sigma n° 587 M). Results are expressed as mmol/L.

Histochemical staining

ALP staining. Fixed cultures (1.5% glutaraldehyde in 0.14 M sodium cacodylate buffer, 10 min) were incubated for 1 h in the dark with a mixture, prepared in Tris buffer pH 10, containing 2 mg/mL of Na-α-naphtyl phosphate and 2 mg/mL of fast blue RR salt; the incubation was stopped by rinsing the samples with water. The presence of ALP was identified by a brown-black staining.

Phosphate deposits (von Kossa assay). Fixed cultures were covered with a 1% silver nitrate solution and kept for 1 h under UV light. After rinsing, a 5% sodium thiosulphate solution was added for 2 min and cultures were washed again. Phosphate deposits stained black.

Scanning electron microscopy (SEM)

Fixed cultures were dehydrated in graded alcohols, critical-point dried, sputter-coated with gold and analysed in a JeoL JSM 6301F scanning electron microscope equipped with a X-ray energy dispersive spectroscopy (EDX) microanalysis capability (Voyager XRMA System, Noran Instruments).

Statistical analysis

Three separate comparative experiments were performed. In each experiment, six replicates were done in the biochemical evaluation and three replicates in the histochemical and SEM characterization. The pattern of cell behavior was similar in the three experiments and data from a representative experiment are shown. Statistical analysis was done by one-way analysis of variance (ANOVA). The statistical differences between the different groups were determined by the Bonferroni method. *P*-values ≤ 0.05 were considered significant.

Results

Cell morphology and pattern of cell proliferation

HG, HPL and HAB cells presented a similar behavior regarding the morphological changes occurring during cell adhesion and spreading and no apparent differences were observed in the three situations tested. Cells contacted with the culture surface within minutes and expansion of the cytoplasm occurred rapidly. After few hours (6 h), cells began to present an elongated morphology and in 24-h cultures they showed a fibroblast-like appearance with few cytoplasmic expansions and cell-to-cell contact. Fig. 1 shows the SEM appearance of the AA-treated cultures.

HG cells presented a high proliferation rate with a typical parallel alignment and the formation of continuous multilayers, in the three experimental situations. HPL cells treated with AA had a similar appearance. However, post-confluent cultures exposed to AA + βGP and, particularly, to AA + βGP + Dex

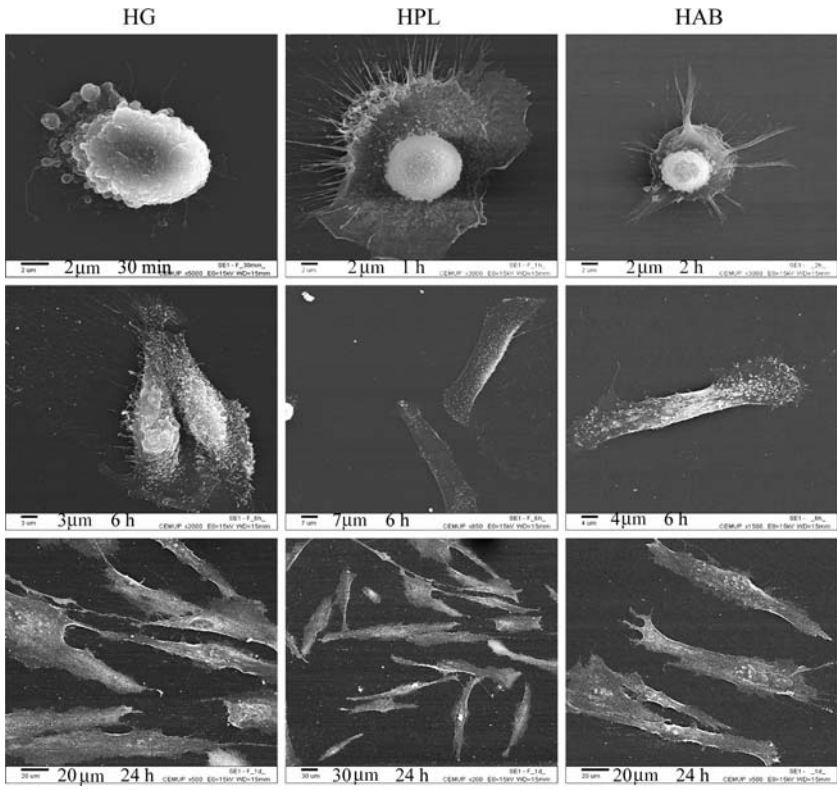

Fig. 1 SEM observation of the attachment and spreading of human periodontal cells during the first 24 h of culture: HG cells (30 min, 6 h and 24 h), HPL cells (1 h, 6 h and 24 h) and HAB cells (2 h, 6 h and 24 h). Cultures treated with AA

showed localized areas of higher cell density that appeared as discrete multilayered structures on long incubation time (4 and 5 weeks) and with a more intense stain for ALP than the bulk of the culture (not shown). HAB cells proliferated to form colonies that grew throughout the culture time giving rise to multilayered three-dimensional structures that further organized themselves in progressively larger nodules, particularly in Dex treated cultures. These structures appeared as clear white spots under phase contrast microscopy and presented a strong positive ALP staining (not shown). Figure 2 shows the appearance of 35-day cultures in the presence of AA + βGP + Dex, as observed by SEM.

Retraction of the cell layer occurred after approximately three weeks, and further cell proliferation was observed in the newly available plastic surface. This event more pronounced in mineralized cultures.

Cell viability/proliferation. ALP and ACP activities

Figure 3 shows the results regarding cell viability/proliferation and ALP and ACP activities of periodontal cells in culture.

HG cells proliferated throughout the 35-day incubation time, with a high growth rate. Cultures presented low ACP activity and negligible levels of ALP, respectively 0.021 ± 0.001 and 0.002 ± 0.001 nmol/min µg protein in AA-treated cultures, at day 21. The presence of Dex caused increased cell proliferation (28% at day 21) and ALP activity (0.003 ± 0.0005 nmol/min µg protein, at day 21); activity of ACP was relatively constant during the culture time and did not peak at day 21 as observed in the other two situations.

HPL cells presented a high growth rate (slightly higher than that of HG cells), that increased in Dex

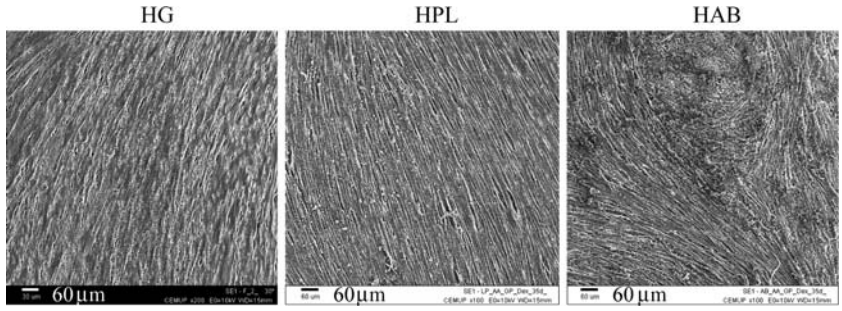

Fig. 2 SEM appearance of the pattern of cell proliferation observed in 35-day cultures treated with AA + βGP + Dex

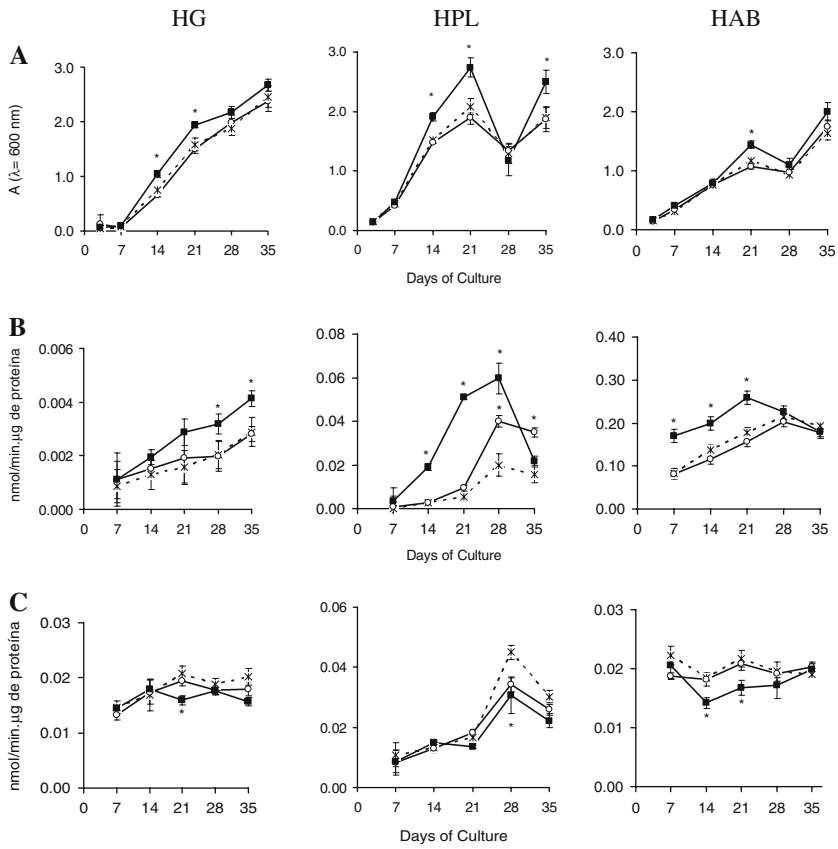

Fig. 3 Cell viability/ proliferation (MTT assay, **A**) and ALP (**B**) and ACP (**C**) activities of HG, HPL and HAB cell cultures grown for 35 days. Cultures treated with AA (×), AA + βGP (○) and AA + βGP + Dex (■). *Significantly different from AA-treated cultures

treated cultures (32% at day 21). MTT reduction increased until day 21, decreased during the fourth week, and increased again in the last week. In AA-treated cultures, ACP activity was higher than that of ALP and enzyme levels increased especially during the fourth week, with maximum values being found around day 28, respectively 0.045 ± 0.002 and 0.020 ± 0.005 nmol/min µg protein. In the presence of AA + βGP, ALP activity was slightly higher than that of ACP at day 28. Additional treatment with Dex resulted in an earlier production of ALP and increased levels (0.060 ± 0.007 nmol/min µg protein, at day 28).

HAB cells showed a pattern of cell proliferation similar to that observed in HPL cells, but a lower growth rate (MTT reduction values were about 70 % lower in AA treated cultures, at day 21), and high ALP activity (0.214 ± 0.011 nmol/min µg protein in AA-treated cultures, at day 21). Cultures treated with AA and AA + βGP presented a similar behavior. The presence of Dex resulted in slight increased cell numbers at day 21 and a significant induction of ALP, with maximum levels attained earlier. ACP activity remained low in the three experimental situations, and the cultures treated with Dex presented the lowest values (0.017 ± 0.001 nmol/min µg protein, at day 21).

Formation of calcium phosphate deposits

The presence of calcium phosphate deposits in the cell cultures was assessed by histochemical staining and SEM observation, Figs. 4 and 5. Also, the concentration of Cai in the medium was measured throughout the 35-day culture time (Fig. 6A), providing quantitative information regarding the calcium deposition in the cell layer; levels reflected changes occurring in intervals of 2–3 days, as the culture medium was totally replaced at each culture medium change.

HG cells showed the formation of multilayered cultures of parallel-oriented cells with abundant non-mineralized extracellular matrix. The EDX spectrum of the cell layer did not show any evidence of Ca and P peaks, von Kossa reaction was negative and the levels of Cai in the culture medium were approximately constant during the incubation time.

HPL cells treated with AA showed a behavior similar to that observed in HG cultures. However,

A

	HG			HPL			HAB		
	21	28	35	21	28	35	21	28	35
AA	–	–	–	–	–	–	–	–	–
AA+βGP	–	–	–	–	–	+/–	–	+	++
AA+βGP+Dex	–	–	–	–	+/–	+	+	++	+++

B

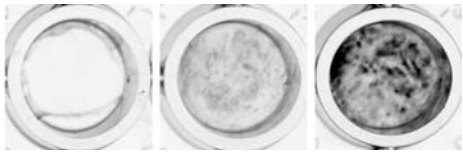

Fig. 4 Histochemical staining of HG, HPL and HAB cell cultures for the presence of calcium phosphate deposits (von Kossa reaction). (**A**) intensity of the staining in 21-, 28- and 35-day cultures; (**B**) appearance of the 35-day cultures treated with AA + βGP + Dex

cultures supplemented with AA + βGP presented the ability to form a very discrete mineralized matrix, and Dex treated cultures showed moderate amounts of mineralized structures in close association with the cell layer. These structures were detected only in 35-day cultures and showed Ca and P peaks on X-ray microanalysis (Fig. 5B). Accordingly, in Dex-treated cultures, levels of Cai in the medium decreased during the last week (Fig. 6A).

HAB cells grown in the presence of AA showed an abundant non-mineralized matrix, whereas those treated with AA + βGP and AA + βGP + Dex presented calcium phosphate deposits within the matrix associated with the three-dimensional nodule-like structures; the Dex treated cultures presented an earlier onset of matrix mineralization and a significantly higher abundance of mineral deposits, as evident from the qualitative (Figs. 4, 5 and 6B) and quantitative assays (Fig.6A). Ability to form a mineralized matrix was much higher than that of HPL cultures (Figs. 4–6).

Discussion

This work compares the long-term behavior of HG, HPL and HAB cells grown in the presence of AA, AA + βGP and AA + βGP + Dex. Cultures were established from healthy explants of periodontal tissues collected from young adults in order to minimize the age-dependent variables [25–28] and experiments were performed in the first subculture to avoid possible changes which may occur during prolonged culture in vitro [24, 29, 30].

AA is an essential nutrient for humans and has a critical role in collagen biosynthesis, the main compo-

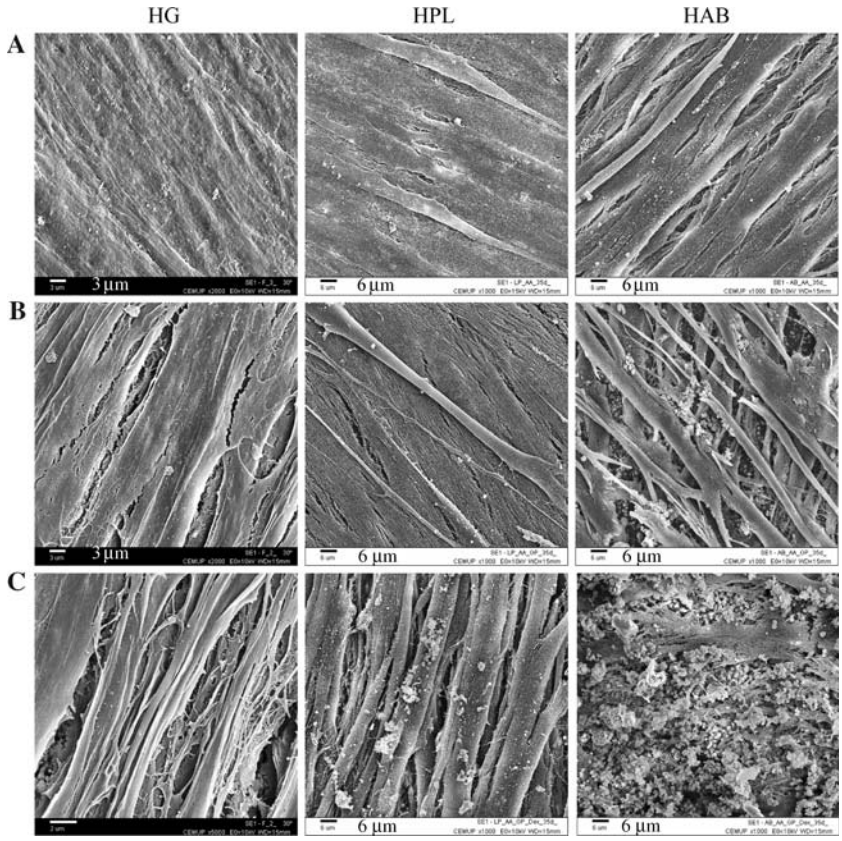

Fig. 5 SEM appearance of 35-day HG, HPL and HAB cell cultures treated with AA (**A**), AA + βGP (**B**) and AA + βGP + Dex (C)

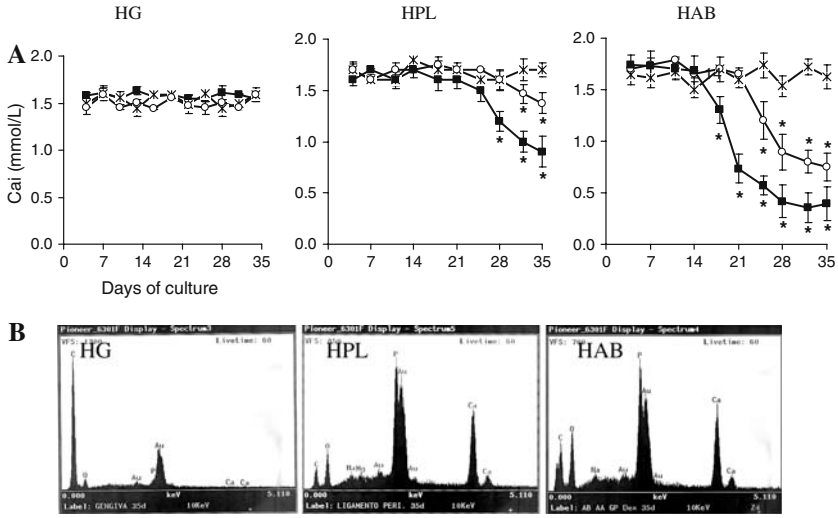

Fig. 6 (**A**) Levels of ionized calcium in the culture medium throughout the 35-day incubation time. Cultures treated with AA (×), AA + βGP (○) and AA + βGP + Dex (■); *significantly different from AA-treated cultures. (**B**) EDS spectra of 35-day HG, HPL and HAB cell cultures treated with AA + βGP + Dex

nent of the extracellular matrix of connective tissues and the most abundant protein in the body [31]. AA is important in normal matrix remodeling activity and also in wound healing and the deficiency of this nutrient is manifested by scurvy, in which a classical clinical feature is severe periodontal pathoses [31]. The mean concentration of AA in gingival crevicular fluid from healthy volunteers is 207 ± 81 μM or 36.46 ± 14.40 μg/mL [32] and thus similar to that used in the present work. AA induces the synthesis of type I collagen in osteoblasts, periodontal ligament cells and gingival fibroblasts and the resulting collagenous extracellular matrix is reported to be involved in the phenotypic modulation of these cell types [33–37].

βGP is routinely added to osteoblast-like cell cultures to promote calcium phosphate deposition in the extracellular matrix [38]. The mechanism by which βGP induces mineralization is closely linked to the high ALP activity of these cultures; this compound is rapidly hydrolyzed by ALP to produce high local levels of phosphate ions providing the chemical conditions for mineral deposition [38–40]. In the present work, cultures were treated with 10 mM βGP, the concentration normally used in in vitro mineralization studies [39, 40]. However, it has been suggested that this concentration of βGP may cause non-biological precipitation of calcium phosphate, [41] a process apparently influenced by the amount of extracellular matrix present in the cultures [42]. In the present work, the addition of βGP resulted in the formation of calcium phosphate deposits in HPL and HAB cultures in close association with the extracellular matrix, suggesting a cell-mediated process. Several studies showed that βGP induces osteogenesis but also affects cell growth parameters and/or ALP activity [38] and, in the present study, HPL and HAB cells responded in a diverse way to βGP, respectively with increased and no effect in ALP activity. This observation may eventually be related to differences in the ratio osteoblastic/non-osteoblastic cells present in the two cell populations and the state of differentiation of committed osteogenic cells, because ALP is a differentiation marker [33, 34].

Dex is a synthetic glucocorticoid frequently used as an in vitro osteoinductive agent, due to its established positive effect on the proliferation and/or differentiation of osteoprogenitor cells in several osteoblast-like cell culture systems [22, 43–47]. The present results showed that the addition of Dex resulted in increased cell proliferation and ALP activity in the three periodontal tissues and induction of matrix mineralization in HPL and HAB cultures. In addition, the positive effect in ALP activity was accompanied by a decrease in ACP levels, suggesting a higher number and/or differentiation of the osteoblast-like cell population in Dex-treated cultures. It has been reported that, in osteoblastic cell cultures, changes in the extracellular matrix in response to Dex favor the obtention of an osteoconductive environment [33, 34, 37]. In addition, studies performed in fibroblastic cells, including HG and HPL cells, showed that Dex inhibits collagenase-1 expression [37] and this event results in decreased matrix turnover and favor, in the case of HPL cells, the accumulation of a collagenous matrix that allows their osteoblastic differentiation [37].

Despite the existence of extensive inter- and intra-population heterogeneity in HG, HPL and HAB cells [13–16], it is clear from the results of the present study that the three cell populations presented distinct characteristics in terms of cell growth and functional activity, regardless the experimental situation used.

HG cell cultures presented a high proliferation rate, synthesis of low levels of ACP and negligible levels of ALP and formation of a non-mineralized extracellular matrix. This is in line with the in vivo behavior of this cell phenotype with the highly proliferative gingival fibroblasts having the task of producing and maintaining the soft tissue lamina propria of the gingiva [1]. It is also in agreement with studies showing that normal connective tissue shows little ALP activity and other bone-associated macromolecules [1, 48, 49] and with results observed in cultured human gingival fibroblasts [2–9, 50]. The presence of some ALP activity in gingival fibroblasts may be related to their anatomical positioning, i.e. close proximity to hard tissues [1, 48], with the possibility that a small subpopulation of these cells may have the ability to express some of the bone-associated proteins. It has been reported that gingival fibroblasts undergo phenotypic modulation as their microenvironment changes [51] and express elevated ALP activity during wound healing and chronic inflammation [52, 53], apparently related to alterations in the composition of the extracellular matrix [36, 54].

HPL cells were highly proliferative and significantly affected by the culture conditions. AA-treated cells expressed a phenotype similar to that observed in HG cultures, but the presence of βGP and, particularly, Dex resulted in a significant increase of the osteoblastic markers. This suggests that HPL in culture includes a proportion of cells showing osteoblast-like properties, with an osteogenic response to appropriate stimulation. This is in agreement with a variety of studies showing that periodontal ligament express ALP and other bone-associated molecules as osteopontin, osteocalcin and bone sialoprotein constitutively in vivo [55–57] and in vitro [7, 9–12]. In addition, treatment of cultured cells with Dex promotes increased expression of osteoblastic markers and the production of a mineralized tissue in the presence of βGP [7, 9, 10, 12, 37, 38]. The ability of periodontal ligament cells to express an osteogenic phenotype was also shown in vivo following systemic treatment with biphosphonates [59]. This is in line with the in vivo situation where periodontal ligament has the ability of producing, maintaining and remodeling both soft and hard periodontal tissues, having a role under normal physiological conditions and also in the periodontal regeneration following surgery and disease [1, 15, 60]. Related to this, it was reported that regenerating HPL cells express elevated ALP levels and increased bone-nodule forming ability as compared to non-regenerating HPL cells and that these osteoblastic markers were further stimulated by Dex [61, 62]. This and other studies suggest that differences in the amount and the composition of the extracellular matrix in normal and regenerative tissues resulting from environmental changes (for instance, the presence of bioactive molecules related to the healing process) may have an important role in the phenotype modulation of HPL cells [37, 63, 64].

HAB cells showed a moderate cell growth rate, production of high levels of ALP and comparatively low levels of ACP, and the formation of abundant three-dimensional mineralized structures in the presence of a source of phosphate ions (βGP). Cultures presented a pattern of cell proliferation/differentiation that is in agreement with the established model for the development of the osteoblastic phenotype [34] and similar to that previously described for this culture system [24, 46, 65] and other animal and human osteoblastic cell systems derived from bone marrow and trabecular bone from different skeletal sites [22, 43, 45, 47]. Alveolar bone is a very dynamic tissue, undergoing constant renewal in response to mechanical, nutritional, and hormonal influences [1], and the behavior of the cultured cells appears to reflect the in vivo situation.

A comparison of the three periodontal tissues regarding cell proliferation revealed that HG and HPL cells showed a higher growth rate as compared to HAB cells, which may be related to the more differentiated nature of these specialized cells [1]. The decrease in the MTT reduction observed during the fourth week in HPL and HAB cell cultures appears to be related to the retraction and the loosening (during medium change) of the cell layer that occurred after three weeks. This process was more pronounced in mineralized cultures and resulted in newly available culture surface that allowed for further cell proliferation, explaining the increase in the MTT reduction observed in the last week. Several studies assessed the relative proliferation rates of gingival and periodontal ligament cells and showed higher [7], similar [2, 8] and lower [4] values for the late cell type. The differences may be explained in part by the existence of extensive heterogeneity in gingival and periodontal ligament cells, as illustrated by the variation in proliferation rates between and within mass cultures and clones derived from these two tissues [5, 49] and also from differences in the experimental conditions used.

The three periodontal tissues also differed markedly in terms of the ability to synthesize ALP and ACP, the pattern of enzyme production and the osteogenic potential. Enzymatic activity was very low, moderate and high, respectively in HG, HPL and HAB cells. In addition, in HG cells, levels increased slowly or remained approximately constant during the culture

time, whereas in HPL and HAB cells a significant increase occurred during the third/fourth week, suggesting a shifting of the cells to a more differentiated stage [34]. Ability to form a mineralized matrix was absent in HG cultures, moderate in HPL cultures and ready in HAB cultures. Gingival cells have been repeatidily shown to express very low levels of ALP and bone associated proteins as compared to periodontal ligament [1–3, 6, 7, 9, 10, 49, 50] and alveolar bone [1, 9, 66, 67] cells. On the other hand, although periodontal ligament exhibit a percentage of cells expressing osteoblastic markers, a variety of studies suggests that they do not normally express the full repertoire of differentiation associated proteins in osteogenesis, as compared to alveolar bone, namely late osteoblastic markers and production of a mineralized tissue [15, 49, 66].

The marked differences found in HG, HPL and HAB cells regarding growth rate and functional activity reflect the different differentiation repertoire of the periodontal cells that critically determine their physiological and regenerative roles [15, 17, 60]. Also, they support the need of guided tissue regeneration to regenerate bone in osseous defects. This approach uses a membrane to create a space between the bone defect and the membrane which facilitates osteoprogenitor cells originating from remaining alveolar bone and periodontal ligament to repopulate and regenerate the defect without the interference of other competitively cell types, namely the highly proliferative gingival fibroblasts [18].

Conclusions

Long-term HG, HPL and HAB cell cultures presented distinct characteristics, regardless the experimental situation. HG cell cultures showed a high proliferation rate with the production of an abundant non-mineralized matrix. HPL cell cultures expressed an osteoblastic phenotype upon appropriate stimulation. HAB cell cultures presented the typical behavior of an osteoblastic cell system with the ready expression of an osteogenic phenotype. βGP and Dex allowed the modulation of the proliferation/differentiation behavior of periodontal cells within the proposed physiological and regenerative capabilities of these cell types. HG, HPL and HAB cells maintained their distinct characteristics in vitro and were sensitive to the presence of bioactive compounds, suggesting a potential utility of this model to perform research in periodontal regenerative procedures.

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