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ORIGINAL ARTICLE

In vitro comparison of chlorhexidine and povidone–iodine on the long-term proliferation and functional activity of human alveolar bone cells

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Abstract This work reports the behaviour of osteoblastic human alveolar bone cells (first subculture) in the presence of chorhexidine (CHX) and povidone–iodine (PI). Short contact (2 min) of 24-h cultures with CHX, at 0.12 and 0.2%, and PI, at 5 and 10%, caused cell death within minutes; contact with 1% PI resulted in loss of the elongated characteristic cell shape. Cell adhesion was adversely affected at concentrations higher than 5×10^{-5}% CHX or 0.05% PI. Long-term exposure to CHX at 10^{-5} and 10^{-4}% or PI at 10^{-4}% had little effect on cell growth and caused an induction in the synthesis of alkaline phosphatase (ALP). Concentrations of CHX and PI similar and higher than, respectively, 5×10^{-4}% or 0.05% caused dose-dependent deleterious effects. CHX affected mainly the cell growth, whereas the effects of PI were observed mostly in ALP production and matrix mineralization. Considering the levels of CHX and PI used routinely in the oral cavity, results suggest that CHX has a higher cytotoxicity profile than PI. This observation might have some clinical relevance regarding the potential utility of PI in the prevention of alveolar osteitis.

Keywords Chlorhexidine · Povidone–iodine · Human alveolar bone cells · Long-term exposure · Cytotoxicity profile · Dose-effects

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Introduction

Alveolar osteitis is an important postoperative complication that is especially prevalent after the extraction of mandibular third molars, with reported incidence rates ranging from 20 to 30% [1, 12]. Risk factors include bacterial infection, trauma to bone during surgery, smoking, inadequate wound irrigation and poor oral hygiene [1, 12]. The duration of the alveolar osteitis depends on the severity of the disease but usually ranges from 5 to 10 days [12]. The delayed healing at the extraction site is characterized by the premature loss or necrosis of the blood clot, exposure of the underlying bone and moderate to severe postoperative pain [12, 35]. Clinical and laboratory studies have shown the significance of locally increased fibrinolytic activity with subsequent loss of blood clotting in the pathogenesis of alveolar osteitis [11, 12]. The role of oral bacteria in clot fibrinolysis is thought to be significant [12, 32], and prevention of alveolar osteitis has focused on systemic and topical antimicrobial therapies [12, 46].

Chlorhexidine (CHX) is the agent most extensively used and tested, having a broad activity spectrum against oral pathogens, good tolerability and absence of resistance development [42]. CHX is used at 0.12 and 0.2% as a mouthrinse agent and as a local irrigant, reducing significantly the quantity of oral microbial populations and the incidence of alveolar osteitis [9, 12, 13, 22]. CHX exhibits substantivity, which accounts for its efficacy because the slow release, from the tooth surface and/or soft tissue after the application, maintains antimicrobial activity for extended periods [42]. Therefore, oral tissues are exposed to relatively high concentrations for short periods and to progressively lower levels for extended

periods, respectively during and between applications. Related to this, several studies have reported that CHX has cytotoxic effects on mammalian cells at the conditions used clinically. Elsen [23] showed that rinsing with 0.12% CHX, twice daily during 18 days, caused DNA alterations on epithelial cells and linphocytes. Furthermore, it is reported that CHX is able to induce primary DNA damage in leukocytes and in oral mucosal cells in rats treated with 0.12% CHX twice daily during 8 days [38]. In addition, in cultured cells, cytotoxicity of CHX has been shown for blood cells [25], keratinocytes [21, 48], fibroblasts [4, 16–18, 33, 36, 40, 50], osteoblasts and osteoclasts [10] and macrophages [41].

Povidone–iodine [polyvinylpyrrolidone–iodine complex (PI)] is an iodofor with a broad spectrum and high antimicrobial activity, low potency for developing resistance and adverse reactions and low financial cost [26]. In the oral cavity, it is used at levels of 1 to 10% (mainly, 1%) in several conditions. PI has been used to decrease postoperative bacteraemia after oral surgery [37], and the American Dental Association and the American Heart Association have suggested its use for subgingival irrigation in the prevention of bacterial endocarditis [20]. The utility of PI has also been reported in periodontal therapy [14, 26, 28, 39, 44], in controlling the incidence of new caries lesions in children at risk of developing extensive caries [2, 30], but little interest has been shown on the use of this antiseptic in the prevention of alveolar osteitis, with only few reported studies carried out more than a decade ago [31, 47]. Like other antiseptics, PI has a nonselective mechanism of action, and the high concentrations used in the oral cavity may adversely affect the host cells. In vitro studies reported deleterious effects in skin and lung fibroblasts [5], gingival fibroblasts [50], keratinocytes [49], embryonic chick osteoblasts [29], canine embryonic fibroblasts [40] and murine calvaria derived bone cells [10].

Alveolar bone cells have not been tested for the toxic effects of CHX and PI. In addition, information regarding the activity of CHX and PI in osteoblast cells is sparse. The published results address animal cells, namely, murine calvarial cells [10] and embryonic chick osteoblasts [29], and most of the studies deal with high levels and short exposures. This work reports the behaviour of human alveolar bone cells in the presence of CHX and PI in several experimental conditions, namely, short contact with high concentrations, exposure during the cellular adhesion and long-term treatment with low levels. The effect of the antiseptics was characterized with regards to cell morphology, cell proliferation and functional activity. Clinical relevance of the results is discussed in terms of differences in the cytotoxicity profile of CHX and PI.

Materials and methods

Cell culture

Alveolar bone fragments were obtained in oral surgery procedures from four young adult patients (mean age of 34 years) without any bone metabolism pathology. Informed consent to use this biological material, which would be otherwise discarded, was obtained. Four independent cell cultures were established (each one from a different patient), following the procedure described below. Alveolar bone fragments were washed extensively with α-minimal essential medium (α-MEM), minced into small pieces and cultured in α-MEM containing 10% fetal bovine serum, 0.0025 mg/ml fungizone and 0.05 mg/ml gentamicin. Cell growth from the bone fragments was observed after approximately 10–15 days, and primary cultures were maintained until near confluence. First-passage cells (obtained from the primary culture upon enzymatic treatment, 0.4 mg/ml trypsin in 0.25 mg/ml EDTA) were cultured at 10^4 cell/cm^2 in the absence (control cultures) and in the presence of CHX or PI. Cultures were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2, and culture medium was changed twice a week.

Exposure to the antiseptics

First-passage alveolar bone-derived cells, obtained as described above, were exposed to the antiseptics in the following experimental conditions:

(1) Short exposure to high levels. The cell suspension was allowed to adhere to the culture plate for 24 h. At this stage, cells were exposed to CHX (1.2 and 2 mg/ml; 0.12 and 0.2%) or PI (10, 50 and 100 mg/ml; 1, 5 and 10%) during 2 min. Cultures were washed twice with phosphate buffer saline and observed immediately by phase contrast microscopy.

(2) Exposure during the cellular adhesion. The cell suspension containing CHX (0.0001 to 0.05 mg/ml; 10^{-5} to 0.005%) or PI (0.01 to 10 mg/ml; 0.001 to 1%) was seeded and allowed to attach for 24 h. Cell morphology was evaluated at 2, 6 and 24 h.

(3) Long-term exposure. The cell suspension was allowed to attach, and cells were grown for 72 h for optimal logarithmic growth. At this stage, the culture medium was changed to a medium containing CHX (0.0001 to 0.01 mg/ml; 10^{-5} to 0.001%) or PI (0.001 to 5 mg/ml; 10^{-4} to 0.5%), and cells were exposed to the antiseptics until day 35. The antiseptics were renewed in the culture medium at each medium change, twice a week. Cultures were performed in the presence of 0.05 mg/ml ascorbic acid, 10 mM β-glycerophosphate

and 10 nM dexamethasone and were characterized throughout the 35-days incubation time for cell viability/proliferation, total protein content, alkaline phosphatase (ALP) activity and ability to form calcium phosphate deposits in the extracellular matrix.

The concentration range of CHX and PI tested was based on the levels used in routine clinical practice and on a preliminary study, which had demonstrated that lower levels did not affect cell cultures and higher levels caused rapid cell death, at the experimental conditions used.

Controls (cells cultured in the absence of the antiseptics) were performed for all the experimental situations tested.

Culture characterization

Control and antiseptic-exposed alveolar bone cells were observed routinely by phase contrast microscopy. Additionally, cell growth and function were assessed as follows:

Cell viability/proliferation and total protein content MTT assay—reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a purple formazan product by viable cells—was used to estimate cell viability/proliferation [8]. 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 λ=600 nm on a microplate reader. 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 activity Activity of ALP was determined in cell lysates (obtained by treatment of the cell layers with 0.1% triton) and assayed by the hydrolysis of *p*-nitrophenyl phosphate in alkaline buffer solution (pH 10.3). Hydrolysis was carried out for 30 min at 37°C, and the *p*-nitrophenol formed was measured at λ=405 nm. Results are expressed as nanomoles of *p*-nitrophenol produced per min per cm^2 (nmol/min.cm^2). Enzyme activity was also normalized by total protein content and expressed as nmol/min.μg protein.

ALP staining Fixed cultures (1.5% glutaraldehyde in 30 mg/ml 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 fast blue RR salt (Sigma F-0500); the incubation was stopped by rinsing the samples with water. The presence of ALP was identified by a brown-black staining.

Calcium and phosphate deposits Alizarin red and von Kossa assays were used to estimate, respectively, calcium and phosphate deposits in the cell layer. For calcium staining, fixed cultures were covered with a 10 mg/ml S alizarin sodium solution (0.28 mg/ml in NH_4OH), pH=6.4, for 2 min and then rinsed with water and acid ethanol (ethanol, 0.01% HCL). Calcium deposits stained red. For phosphate staining, fixed cultures were covered with a 10 mg/ml silver nitrate solution and kept for 1 h under UV light. After rinsing, a 50 mg/ml sodium thiosulphate solution was added for 2 min, and cultures were washed again. Phosphate deposits stained black.

Scanning electron microscopy (SEM) assay Fixed cultures were dehydrated in graded alcohol (70, 80, 2×90 and 99.8%), critical-point dried, sputter-coated with gold and analysed in a JEOL JMS 630 1F scanning electron microscope equipped with X-ray energy dispersive spectroscopy voyager XRMA system (Noram Instruments).

Statistical analysis

Four independent experiments were performed, with the cultures being established from different patients. In each experiment, six replicates were done in the biochemical evaluation and three replicates in the histochemical and SEM characterization. The pattern of cell behaviour regarding the dose and time effects of CHX and PI on alveolar bone cells was similar in the four experiments. Data from a representative experiment are shown. Statistical analysis was done by one way analysis of variance. The statistical differences between control and treated cultures were determined by the Bonferroni method. P values$\leq$0.05 were considered significant.

Results

Short exposure to high levels

Exposure of 24-h cultures to CHX (1.2 and 2 mg/ml) or PI (50 and 100 mg/ml) during 2 min resulted in almost immediate cell destruction. PI, at 10 mg/ml, caused a rounding up of the cells followed by progressive detachment from the culture plate.

Exposure during the cellular adhesion

Attachment of alveolar bone cells to the culture substratum occurred within minutes after plating; cytoplasm expansion started immediately and, after 2 h, it was relatively spread with the bulge of the nucleus apparent. Cell morphology became progressively elongated, and a flattened fibroblastic appearance was visible at 24 h.

Exposure to levels of CHX up to 0.005 mg/ml or PI up to 0.5 mg/ml did not adversely affect the attachment and the morphological changes occurring during the adhesion process. Higher concentrations caused significant dose- and time-dependent effects with a decrease in cytoplasmic volume and number of attached cells. In the presence of 0.05 mg/ml CHX, the few adherent cells were unable to perform the normal cytoplasmic spreading process (cultures with 2 and 6 h) and detached progressively from the flask surface; 24-h cultures presented mostly cellular debris and dead cells. Exposure to10 mg/ml PI resulted in rapid cell death, and 2-h cultures showed only cellular debris.

Figure 1 shows the appearance of 24-h control and antiseptic-treated cultures, observed by optical and scanning electron microscopy.

Long-term exposure

Cellular proliferation

In control cultures, cellular proliferation increased exponentially until day 21, decreased during the fourth week and increased again afterwards. Microscopic observation showed a characteristic pattern of cell growth with the formation of localized areas of high cell density that formed nodule-like structures on long incubation times.

CHX, at 0.0001 mg/ml, caused a small positive effect on cell growth; higher levels (0.001, 0.005 and 0.01 mg/ml) resulted in a dose-dependent inhibitory effect. Cells seemed to progressively adapt to the presence of 0.005 mg/ml, as suggested by the increase in the proliferation observed during the last week. Exposure to 0.01 mg/ml resulted in a strong cytotoxic effect. PI, at 0.001 and 0.01 mg/ml, did not affect cell proliferation. Exposure to higher levels caused a dose-dependent inhibition. Progressive adaptation to the presence of 2 mg/ml occurred, but cultures exposed to 5 mg/ml presented very low MTT reduction values.

Figures 2 and 3 show, respectively, the results regarding the MTT assay and the pattern of cell growth of control and antiseptic-treated cultures.

ALP activity

Alveolar bone cell cultures produced high levels of ALP. Activity of the enzyme increased until day 21 and decreased afterwards.

The continuous exposure to 0.0001 and 0.001 mg/ml CHX caused an evident increase in ALP activity (around 30% at day 21). In the presence of 0.005 mg/ml, activity of the enzyme was similar to that of control cultures. Very low levels of ALP were found in the cultures treated with 0.01 mg/ml CHX due to the almost absence of cell proliferation. PI, at 0.001 mg/ml, caused an increase in ALP activity around day 21, and the presence of 0.01 mg/ml did not affect enzyme production. Exposure to 0.5 and 2 mg/ml resulted in a significant dose-dependent decrease in the levels of the enzyme.

Results are shown in Fig. 2.

Formation of calcium and phosphate deposits

Human alveolar bone cell cultures showed the presence of mineralized calcium phosphate deposits after 3 weeks of incubation, clearly identified in 28- and 35-day cultures by positive Alizarin red and von Kossa staining. Mineral deposits were closely associated with the cell layer, as observed by SEM, and contained calcium and phosphorous, as shown by X-ray microanalysis.

Cultures exposed to 0.0001, 0.001 and 0.005 mg/ml CHX presented a similar behaviour, but in the presence of 0.0001 mg/ml increased extent of matrix mineralization was noted. As mentioned above, exposure to 0.01 mg/ml resulted in cell death. PI, at 0.001 and 0.01 mg/ml, did not affect the formation of calcium phosphate deposits. In the presence of 0.5 and 2 mg/ml, matrix mineralization was not observed despite the significant cell proliferation observed in these situations, especially in the cultures exposed to 0.5 mg/ml.

Results are shown in Figs. 4 and 5.

Discussion

The present work reports the behaviour of human alveolar bone cell cultures in the presence of CHX and PI. First-passage cells were cultured in presence of ascorbic acid, β-glycerophosphate and dexamethasone, compounds known to favour the proliferation and differentiation of osteoblast cells [45]. Results showed that the cultures grown in these conditions presented a proliferative phase accompanied by the synthesis of ALP and matrix mineralization after 3 to 4 weeks, following a pattern similar to that observed previously [19, 24, 34] and in agreement with that reported for the development of the osteoblast phenotype [45]. The proliferation/differentiation behaviour of alveolar bone cell cultures was also similar to that observed in vivo during the healing of tooth extraction wounds. In this process, after the replacement of the initial blood clot by the granulation tissue, an active phase of bone cell proliferation and differentiation occurs and bone begins to form between 8 and 12 days [3]. Therefore, the in vitro model used in this study seems to be adequate to study the response of alveolar bone cells to CHX and PI.

In the oral cavity, CHX is used at 0.12 and 0.2% (1.2 and 2 mg/ml) and PI at 1 to 10% (10 to 100 mg/ml). Data regarding the penetration of these antiseptics into the blood

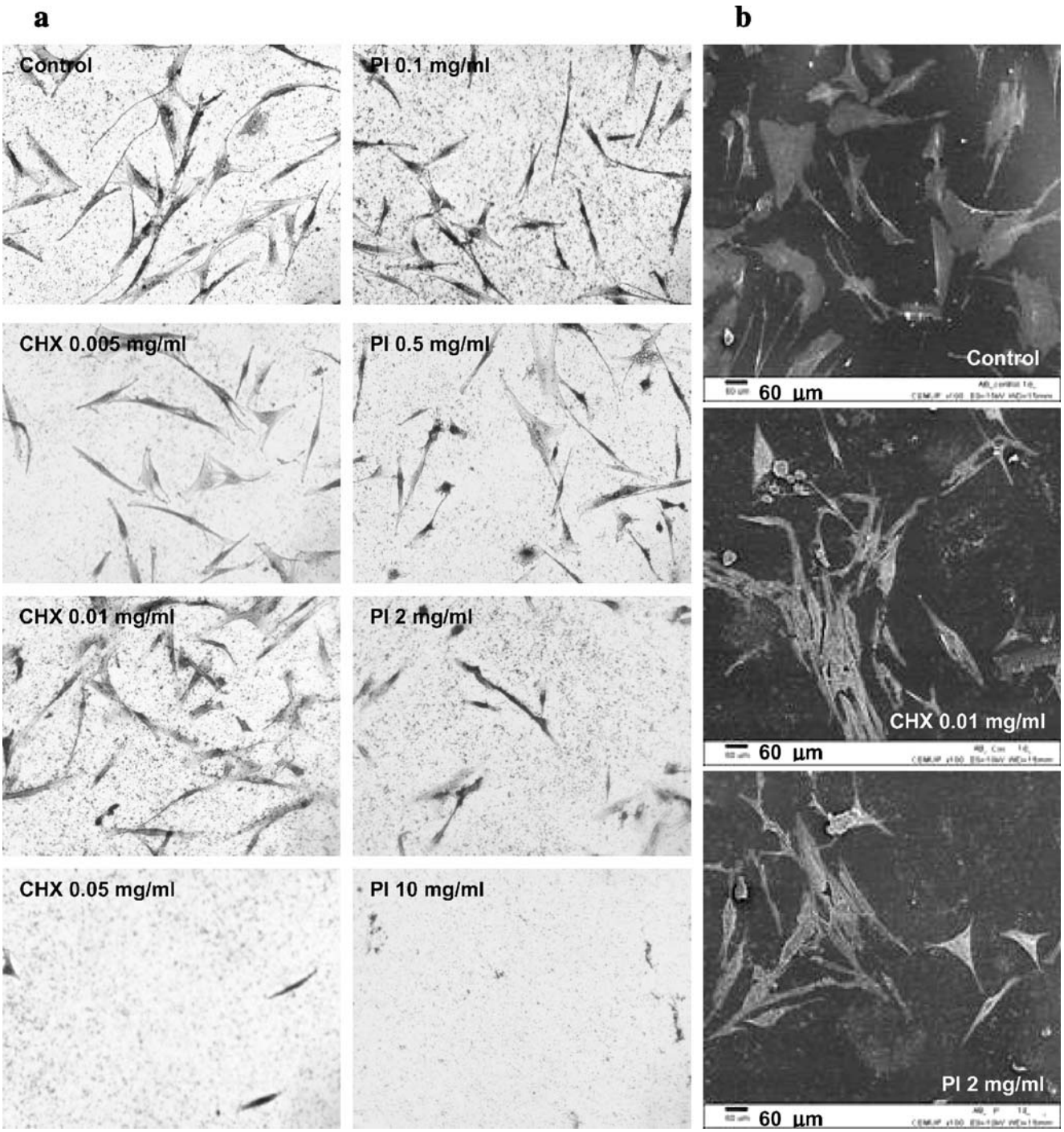

Fig. 1 Human alveolar bone cells cultured for 24 h, observed by optical microscopy after ALP staining, ×100 (**a**) and scanning electron microscopy (**b**). CHX, at levels up to 0.005 mg/ml and PI, at levels up to 0.5 mg/ml, did not cause significant effects on cellular adhesion and morphology. Higher concentrations resulted in a dose-dependent decrease in cytoplasmic volume, number of attached cells and, ultimately, cell death

clot and oral biofilms are not available in the literature. However, considering the loosely nature of these structures, compared to that of the organized tissues, and the clinical outcome achieved with the use of these agents, active antimicrobial levels (close to those present in the oral formulations) might be expected on the underlying bone tissue. Due to the subsequent dilution of the antiseptic in the oral cavity, cells will also contact with much lower levels for extended periods of time. Therefore, in this work, the behaviour of alveolar bone cells was assessed in the presence of high levels of CHX or PI and short exposures and, also, low levels of these agents but long-term contact.

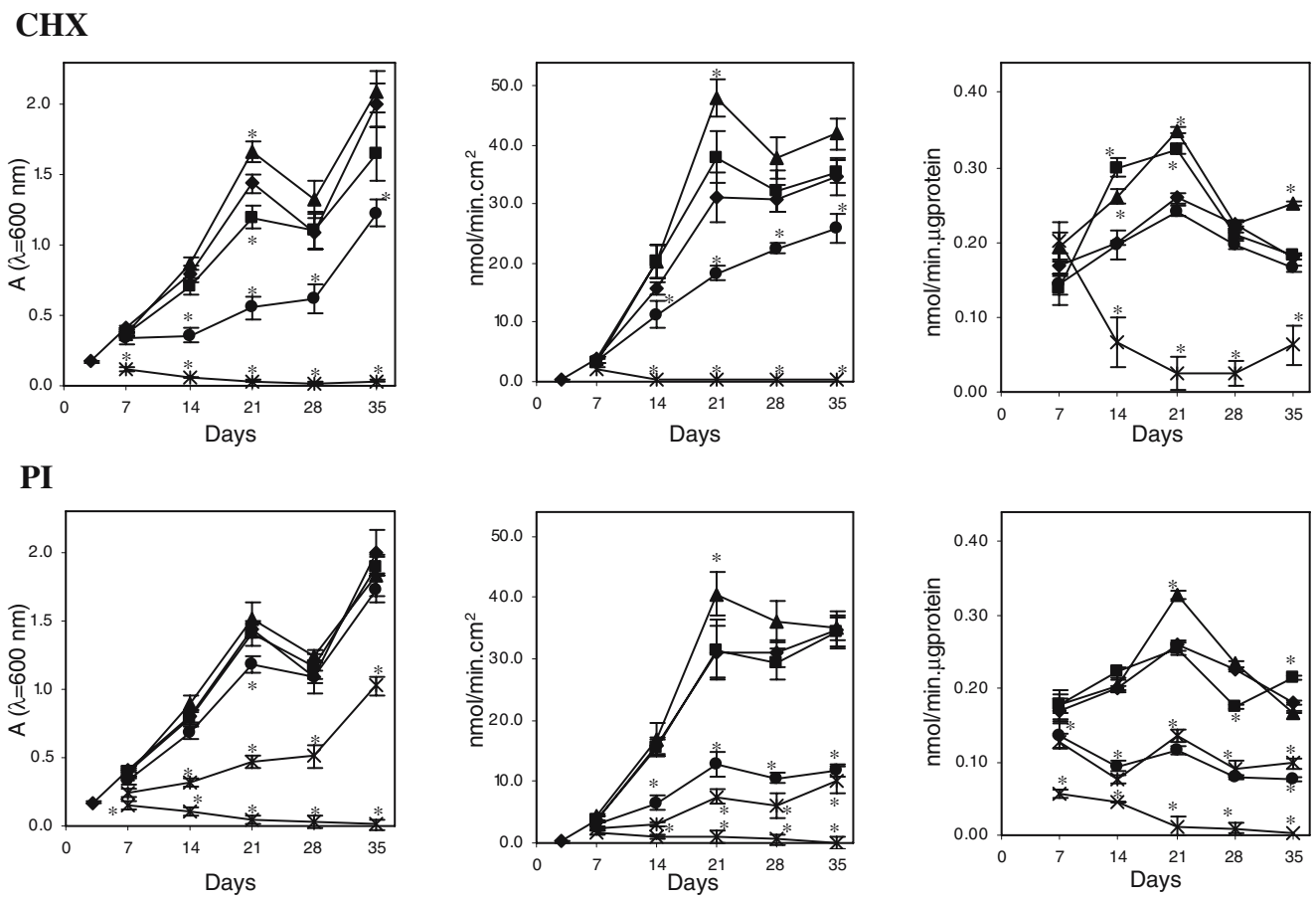

Fig. 2 Cell viability/proliferation (λ=600 nm) and alkaline phosphatase activity (nmol/min.cm^2; nmol/min.µg protein) of human alveolar bone cells cultured for 35 days. Control cultures (*filled diamonds*); CHX-treated cultures: 0.0001 (*filled triangles*), 0.001 (*filled squares*), 0.005 (*filled circles*) and 0.01 (*big asterisks*) mg/ml; PI-treated cultures: 0.001 (*filled triangles*), 0.01 (*filled squares*), 0.5 (*filled circles*) 2 (*big asterisks*) and 5 (*multiplication symbol*) mg/ml. Significantly different from control cultures (*small asterisks*)

The short exposure (2 min) of adherent bone cells to levels of CHX or PI used in the routine clinical practice resulted in almost immediate cell destruction. In addition, the adhesion of alveolar bone cells to the culture substratum was adversely affected at concentrations higher than 0.005 mg/ml CHX and 0.5 mg/ml PI. Osteoblastic cell attachment and spreading to the substratum (in vitro, the culture plate; in vivo, bone surfaces undergoing bone formation) are essential processes for normal cell growth and differentiation [27]; the morphological changes occurring during these events correspond to the reorganization of the cytoskeleton, the structure that plays a role in the

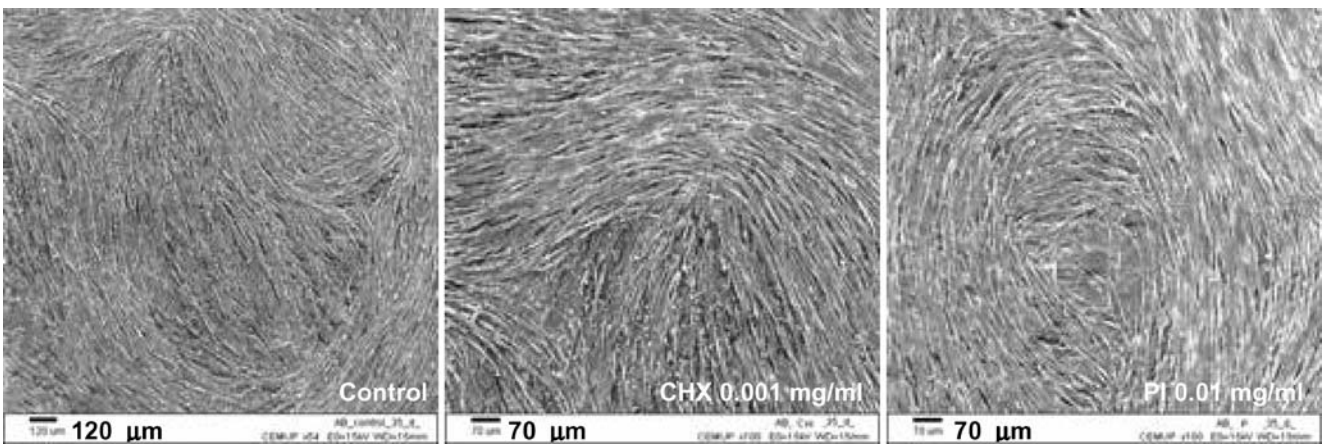

Fig. 3 Human alveolar bone cells cultured for 28 days, SEM observation. Control cultures presented a characteristic pattern of cell growth, with the formation of nodule-like structures. Cultures exposed to CHX, at 0.001 mg/ml, and PI, at 0.01 mg/ml, presented a similar appearance

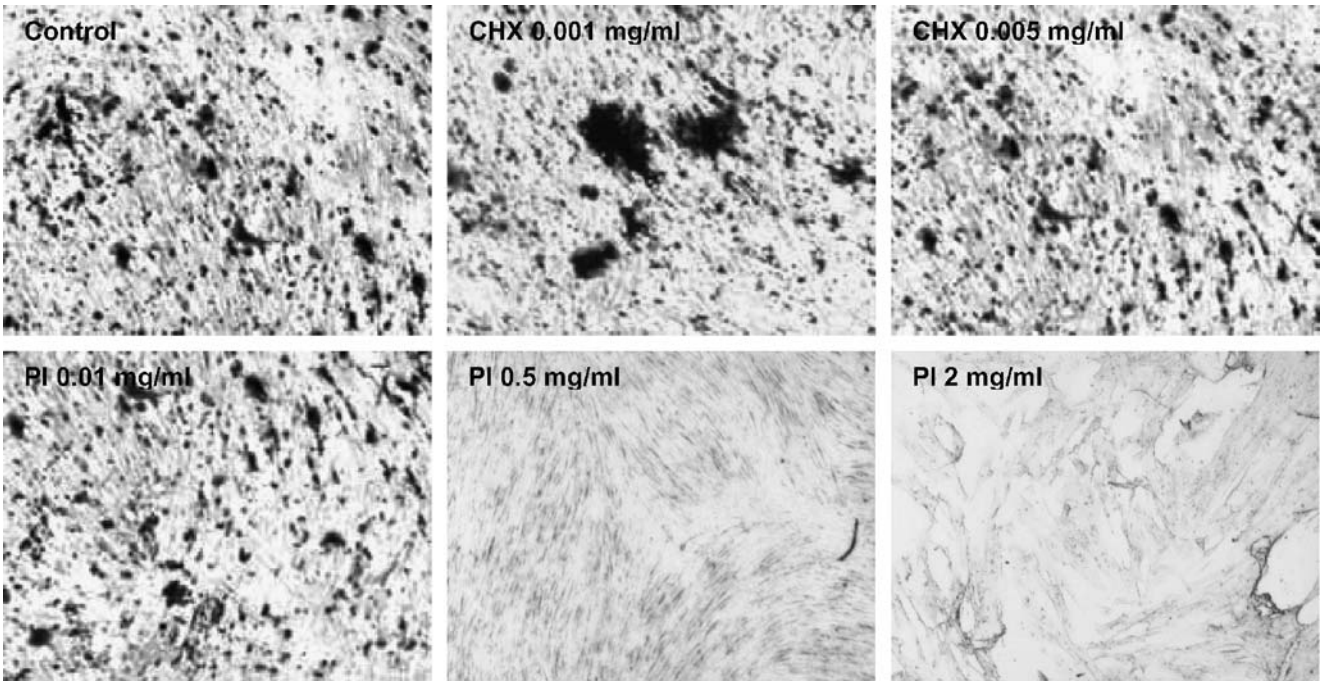

Fig. 4 Human alveolar bone cells cultured for 35 days and stained for the presence of calcium deposits (Alizarin red assay); optical microscopy, ×40. Positive histochemical staining was observed in control cultures and those exposed to 0.001 and 0.005 mg/ml CHX or 0.01 mg/ml PI. In the cultures treated with 0.5 and 2 mg/ml PI, the cell layer presented negative staining for the presence of calcium deposits

control of the cell shape and behaviour [27]. Long-term exposure of adherent bone cells to antiseptic levels lower than those resulting in cell death affected the cell behaviour in a dose-dependent manner. Low concentrations of CHX and PI caused a stimulatory effect in the cellular activity, probably reflecting a general unspecific response to the presence of an aggressive stimulus. Higher levels resulted in negative effects. CHX appeared to cause a more significant deleterious effect on cellular proliferation than in the functional activity, as shown by the lower values for MTT reduction, but similar ALP activity and matrix mineralization observed in the cultures exposed to 0.005 mg/ml, as compared to control cultures. The opposite seemed to occur with PI. Levels of 0.5 and 2 mg/ml caused, respectively, a slight and a moderate decrease on cell growth, but ability to synthesize ALP was greatly reduced; as expected, in these conditions, matrix mineralization was not observed, as high ALP activity at sites of mineralizable matrices is necessary to increase the local phosphate concentrations (in the experimental conditions used, by hydrolysing β-glycerophosphate) for mineral deposition [7].

The behaviour of osteoblastic cells in the presence of CHX and IP was previously analysed in newborn murine calvarial cells [10]. In this study, adherent cells were exposed to CHX (10 and 40 mg/ml) and PI (10 and 100 mg/ml) for 2, 10 and 20 min, and cultures evaluated for the number of viable cells (24 h after the exposure) and number of alkaline positive cells and bone nodules (at day 21). Authors showed a significant dose- and time-dependent inhibitory effect in the measured parameters. In another study, Kaysinger et al. [29] found that a 2-min exposure of embryonic chick osteoblasts to 50 mg/ml PI caused a 30% decline in lactate production (a marker of glycolytic energy metabolism) and a 90% decrease in DNA synthesis. Comparison of these observations with those of the present work is difficult due to differences in exposure conditions, the cell culture system used and the phase of the culture in which the exposure occurred (i.e. cell adhesion, exponential cell growth and confluent cultures). Several studies also reported that CHX and PI affected the behaviour of cultured cells other than osteoblasts. The substrate adherence capacity of macrophages was inhibited in a dose- and time-dependent way by CHX at levels of 0.0012 to 0.12 mg/ml [41], and attachment of human periodontal fibroblasts to pretreated root surfaces with 2 mg/ml CHX during 15 min was also impaired [18]. PI, at 1 and 10 mg/ml, reduced the attachment of skin and lung fibroblasts [5]. Branchet et al. [16] found increased proliferation of human oral fibroblasts exposed to 10^{-4} mg/ml CHX for 3 days. Dose-dependent inhibitory effects of CHX were found in human oral fibroblasts [4, 16–18, 33, 36], skin fibroblasts and keratinocytes [48] and in murine bone cells [10], and cell death occurring within minutes was reported for human gingival fibroblasts exposed to 0.2 and 2 mg/ml CHX [50]. Regarding the in vitro toxicity of PI, deleterious effects were noted in skin and lung fibroblasts [5], skin fibroblasts and keratinocytes

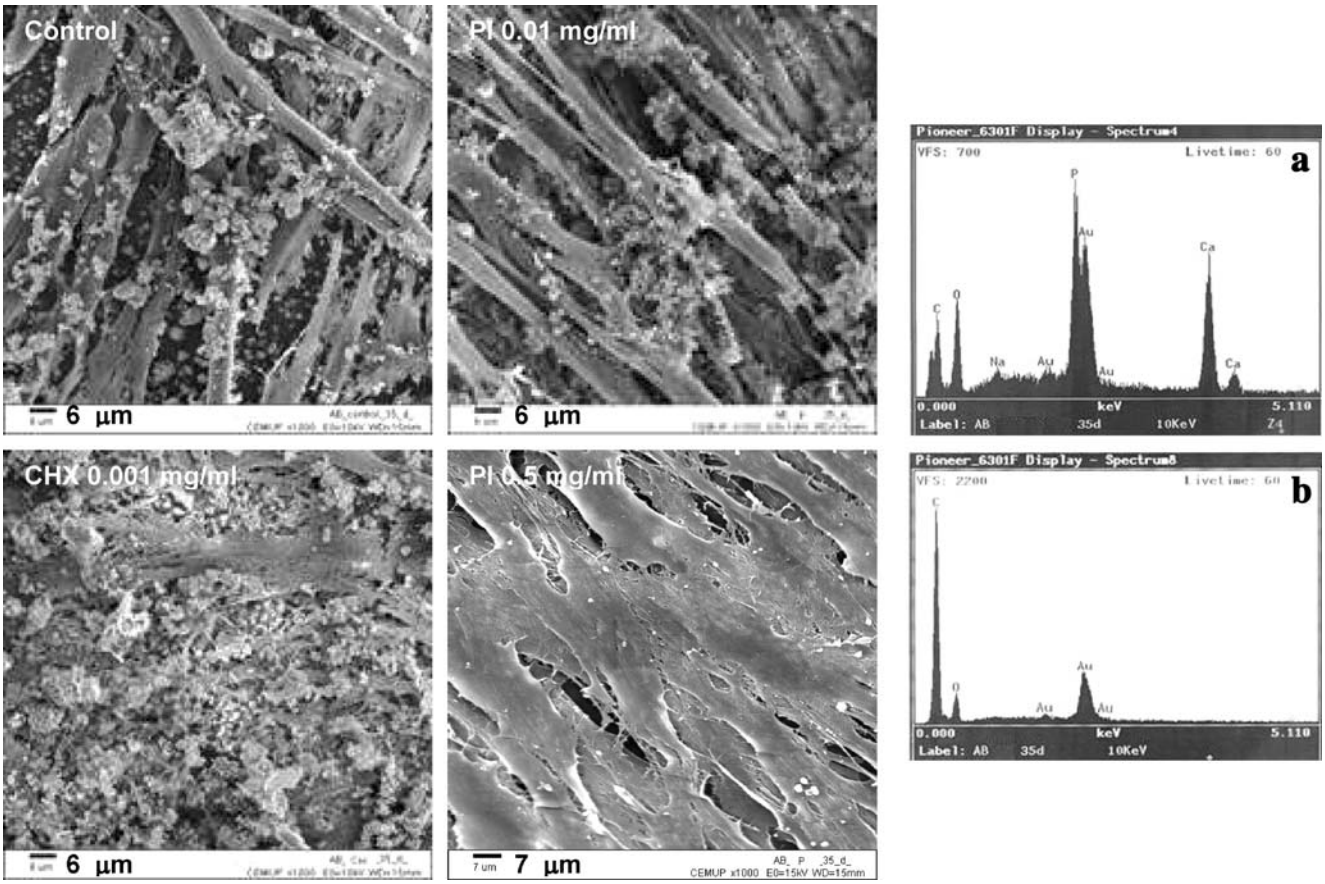

Fig. 5 Human alveolar bone cells cultured for 35 days, SEM observation. Control cultures showed the presence of abundant globular mineralized structures closely associated with the cell layer. **a** Representative EDS spectrum of the mineral structures, showing the presence of Ca and P peaks; **b** EDS spectrum of the cell layer in the cultures exposed to 0.5 mg/ml PI, with absent Ca and P peaks. CHX, at 0.001 mg/ml, caused an evident stimulatory effect in the mineral deposition. PI did not affect matrix mineralization at 0.01 mg/ml but inhibited this process at 0.5 mg/ml

[21], and treatment of human gingival fibroblasts with 10 mg/ml PI resulted in immediate cell death [50].

Experimental and clinical studies using CHX on mucosal-osseous wounds have shown both adverse [6, 43] and beneficial [15] effects on wound healing. In the later work [15], authors assessed the influence of a 1-month period of CHX rinses in the remodelling activity of periodontal tissues adjacent to an extraction wound and demonstrated an increase in alveolar bone density between months 1 and 6 in the treated group as compared to the control group. With regards to PI, there have been no histological studies to assess the impact of this agent in wound healing within the oral cavity. In general, after PI (10%) use, researchers have not reported any impaired healing or untoward systemic or tissue reactions [14, 26, 28, 37, 39, 44]. In the present work, the in vitro toxicity of CHX and PI was shown to be dependent on the concentration and the duration of the exposure. Levels routinely used in the clinical practice resulted in rapid cell destruction, but the prolonged presence of very low levels caused a stimulatory effect in the cellular behaviour; this mechanism, especially in the case of CHX, due to its substantivity and consequent longer stay in the oral cavity [42], may contribute to the overall effect of these agents in wound healing. In addition, in vivo cytotoxicity is expected to be lower than that in cultured cells due to several factors including the tridimensional multilayered tissue organization with the cells being surrounded by abundant extracellular matrix, the drug diffusion to the vascular system, the host defense mechanisms and the complexity of the oral environment with bacterial and other biomolecules competition, as well as the rapid salivary dilution of the antiseptic. However, the present results suggest a relatively low toxicity potential of PI compared to CHX at the levels used in the oral cavity; other studies performed in fibroblasts and keratinocytes showed a similar tendency [10, 21, 40, 50]. This observation may have some clinical relevance, considering that PI is a low cost and highly effective antimicrobial agent. Furthermore, it does not exhibit adverse side effects such as discoloration of teeth and tongue and change in taste sensation [14, 26], as seen with CHX [42]. Results suggest the need for additional studies addressing the eventual

utility of PI in the prevention of alveolar osteitis and also its effect in mucosal-osseous regeneration as compared to CHX.

Conclusion

CHX and PI affected the behaviour of human alveolar bone cells in a dose-dependent manner. CHX, at 1.2 and 2 mg/ml, and PI, at 50 and 100 mg/ml, caused almost immediate cell destruction. Cell adhesion was adversely affected at concentrations higher than 0.0005 mg/ml CHX or 0.5 mg/ml PI. Long-term exposure to CHX, at 0.0001 and 0.001 mg/ml, or PI, at 0.001 mg/ml, caused a stimulatory effect in the functional activity; levels similar and higher than 0.005 mg/ml CHX or 0.5 mg/ml PI resulted in deleterious effects on cell proliferation and function. Considering the levels of CHX and PI used routinely in the oral cavity, results suggest that PI has a lower cytotoxicity profile than CHX.

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