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Original article

Can non-collagenous proteins be employed for the differential diagnosis among fibrous dysplasia, cemento-osseous dysplasia and cemento-ossifying fibroma?

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ABSTRACT

Differential diagnosis among fibrous dysplasias, cemento-ossifying fibromas and cemento-osseous dysplasias is difficult, since there is considerable overlap of histologic features, but also extremely important, since they differ greatly in etiology, clinical behaviour, prognosis and terapeuthic approach. There is no data about the use of immunohistochemistry, a viable and accessible technique, for this purpose. The objective of this study was to investigate, comparatively, the immunohistochemical expression of major non-collagenous proteins (osteonectin [ON], osteopontin [OP], bone sialoprotein [BSP] and osteocalcin [OC]) of mineralized tissue extracellular matrix in 22 cases of fibrous dysplasias, 16 of cemento-ossifying fibromas and 16 of cemento-osseous dysplasias. ON maintained the same expression profile in all cases; the staining for OP was negative in fusiform cells producing cementoid globules and weak, as well as heterogeneous, in high mineralized matrixes; there was negativity for BSP in cementoid globules and in the fusiform cells that produce them, differently from the strong positive expression found in the majority of bone trabeculae and their peripheral cuboidal osteoblasts; and finally, the immuno-reactivity for OC was weak, except in cuboidal osteoblasts and osteocytes. We can conclude that the nature of mineralized structure and the cellular phenotype are much more responsible for variability in immunohistochemical profile than the type of lesion (fibrous dysplasias, cemento-ossifying fibromas and cemento-osseous dysplasias) which makes difficult, at least for a while, the use of these proteins with diagnosis purpose.

1. Introduction

The diagnosis among fibrous dysplasias, cemento-ossifying fibromas and cemento-osseous dysplasias is usually a dilemma due to the considerable overlap of histologic features [1]. Many times, in the absence of good clinical information, the pathologist can only state that a given biopsy is consistent with a benign fibro-osseous lesion or benign mesenchymal odontogenic tumour [2,3]. However, as fibrous dysplasia represents a developmental disturbance, cemento-ossifying fibroma is considered a genuine neoplasm and cemento-osseous dysplasias correspond to reactive phenomena, different therapeutic approaches are required [4,5]. Therefore, generical designations have to be avoided.

Fibrous dysplasias are characterized by cellular fibrous connective tissue interspersed by delicate trabeculae of immature bone that do not connect to each other and appear evenly distributed through the stroma. Progressive maturation can lead to the appearance of osteoid, lamellar bone and peripheral osteoblasts. The cemento-ossifyng fibromas are formed by connective tissue of high cellularity, low collagenization and poor vascularization, interspersed by numerous basophilic globular / curvilinear structures of cementoid calcification and also bone trabeculae, immature or lamellar, surrounded by osteoid and showing uniform dimensions. In incipient cemento-osseous dysplasias, there is more cellular connective tissue, interspersed by a small quantity of calcified material, both irregular trabeculae of immature bone and basophilic, acellular and avascular globules. Mineralized structures are confluent and compatible with bone and cement, this exhibiting basophilic lines and fibrillar eosinophilic periphery. With maturation, the connective tissue becomes more fibrous and the degree of

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mineralization increases, giving rise to large and dense sclerotic masses [6–8].

The discovery of specific immunohistochemical markers that characterize different profiles of expression could help, not only to distinguish these lesions, but also to better understand their etiology, behavior and natural history. Considering that the mineralized component has an important role in the microscopic characterization of these lesions, it is reasonable to study their organic phase constituent proteins, especially by means of immunohistochemistry, a viable and accessible technique, whose application in diagnosis process is usually contributive. The noncollagenous matrix proteins synthesized by cells of osteoblastic lineage could be responsible for defining specific properties that distinguish the various mineralized tissues.

Osteonectin is a phosphorylated glycoprotein, not specific of mineralized tissues, whose roles are greatly affected by the presence or absence of phosphate [9]. In bone, it promotes attachment of hydroxyapatite crystals to collagen fibers, because of its exclusive ability to bind, simultaneously and greedily, to both, using two distinct domains [10]. It seems to be more related to the matrix deposition [11], but it can also act in bone structure mineralization control [12], especially in initial phases, as nucleator of crystal formation and to avoid premature mineralization [13]. It also modulates the proliferation of osteoblastic lineage cells [14], regulating cell cycle, shape, differentiation/activation and cell-matrix interactions [10,12].

Osteopontin is a phosphorylated and sulfated glycoprotein, highly acidic [15,16] and produced by different cellular types. It is present in bone, teeth, kidney, most epithelial lining cells, body secretions (urine, saliva, milk and bile), acute and chronic inflammatory conditions and in various pathologies, associated or not to calcifications [15]. It is important in the wound healing process of soft tissues and in mineralization and remodeling of hard tissues [12,14,15]. Osteopontin acts on fibroblasts and endothelial cells, stimulating adhesion and migration [17,18]. In macrophages, it regulates proliferation, differentiation, endothelial transmigration, interstitial migration, adhesion, function, phagocytosis and survival. In osteoclast, it also participates in modulation of recognition and interaction with αvβ3 integrin, by stimulating cytoskeletal rearrangements [15]. Whereas in cementum the function of osteopontin is not completely understood and appears to be related to the regulation of tissue formation [19]; in bone, osteopontin has a double role: in early events it stimulates osteoblasts adhesion, and in later events (as in remodeling) acts on osteoclasts [20]. It has an essential role in mineralization, as it can bind strongly to hydroxyapatite and should, therefore, regulate calcium-phosphate deposition, both inhibiting (as a physical limiting) and promoting (as nucleator) crystal formation and growth [15].

Bone sialoprotein is an acidic, phosphorylated and sulfated glycoprotein [16,21,22] that represents 10–15% of noncollagenous proteins from mineralized bone matrix and exhibits high affinity for hydroxyapatite [18,21]. It is associated to mineralization, both pathological and of normal bone [18,23], because of the ability to inhibit or nucleate and mediate hydroxyapatite crystal growth, depending on its physical state, if immobilized or in solution [23]. Although BSP seems to have an important role in cementogenesis process [24], its effects are more studied in bone remodeling. During resorption, BSP uses αvβ3 integrin to promote and modulate cellular adhesion and activation, making easier resorptive activity without, however, increasing the number of osteoclasts; whereas during bone formation, it interacts with endothelial cells, stimulating angiogenesis and giving support to vascular invasion process. It also promotes mitosis and differentiation of pre-osteoblasts, increasing the number of active osteoblasts [18]. Serum levels of BSP tend to reflect bone turn-over and are elevated in post-menopause patients, hyperparathyroidism, Paget`s disease, non-treated multiple myeloma, rheumatoid arthritis and also in breast cancer, where BSP is associated to hydroxyapatite crystals formation, that leads to bone metastasis and to a worst prognosis [18,23].

Osteocalcin, also called GLA-bone protein, is a glycoprotein rich in γ-carboxiglutamic acid, which exhibits high homology with GLA-protein of matrix, suggesting a common ancestral [10]. In mineralization, the affinity with hydroxyapatite is only relatively high [10,22], but it is sufficient to permit that osteocalcin act as an inhibitor of crystal growing [14,22], blocking the transition from bruxita to hydroxyapatite and inhibiting the precipitation of this from supersaturated solutions. In this way, the protein plays important role in regulation of crystal maturation [9] and in the maintenance of matrix, being apparently unnecessary in the initial phase of mineralized process [14]. When stimulated by D3 vitamin, osteocalcin inhibits collagen synthesis and promote bone reabsorption, signalizing for osteoclasts [10,25] and acting as chemoattractant for their precursors [14,25].

In the present study, the immunoprofiles of osteonectin (ON), osteopontin (OP), bone sialoprotein (BSP) and osteocalcin (OC) in fibrous dysplasias, cemento-osseous dysplasias and cemento-ossifying fibromas were analyzed, aiming to investigate a possible use of them in differential diagnosis, by means of immunohistochemistry. As far as we know, there is no similar study in the literature.

2. Material and methods

Fifty-four cases were retrieved from the files of Oral Pathology Department of the University of Sao Paulo – Brazil. Twenty-two cases had been diagnosed as fibrous dysplasia, sixteen as cemento-ossifying fibromas and sixteen as cemento-osseous dysplasias. The diagnoses were checked by three experienced examiners. Cases had to meet the literature criteria already mentioned in introduction and also illustrated by photomicrographs (Fig. 1A–C). The study was approved by Ethics Committee of São Paulo University (nº 42/02; protocol 65/02).

For immunohistochemical reactions, 3-μm sections were obtained from 10% neutral formalin-fixed, paraffin-embedded lesional tissues. They were mounted on slides coated with 3-aminopropyltriethoxy-silane (Sigma Diagnostics, St. Louis, MO, USA), deparaffinized with xylene and rehydrated in descending graded alcohols. To block endogenous peroxidase activity, after rinsing with distilled water, the sections were exposed, for 30 min, to 1:1 dilution of 3% hydrogen peroxide in methanol. They were rewashed with distilled water, immersed three times of 5 min in buffered TRIS-HCL, pH 7.4 and then incubated with normal swine serum (Dako, Copenhagen, Denmark), in moist chamber, for 50 min, to reduce background staining, followed by a new cycle of three immersions in buffered TRIS-HCL.

The primary polyclonal antibodies, raised in rabbits against human

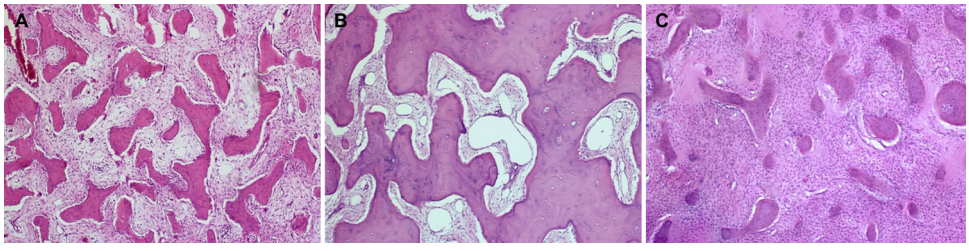

Fig. 1. Representative photomicrographs of fibrous dysplasia **(A)**, cemento-osseous dysplasia **(B)** and cemento-ossifying fibroma **(C)**.

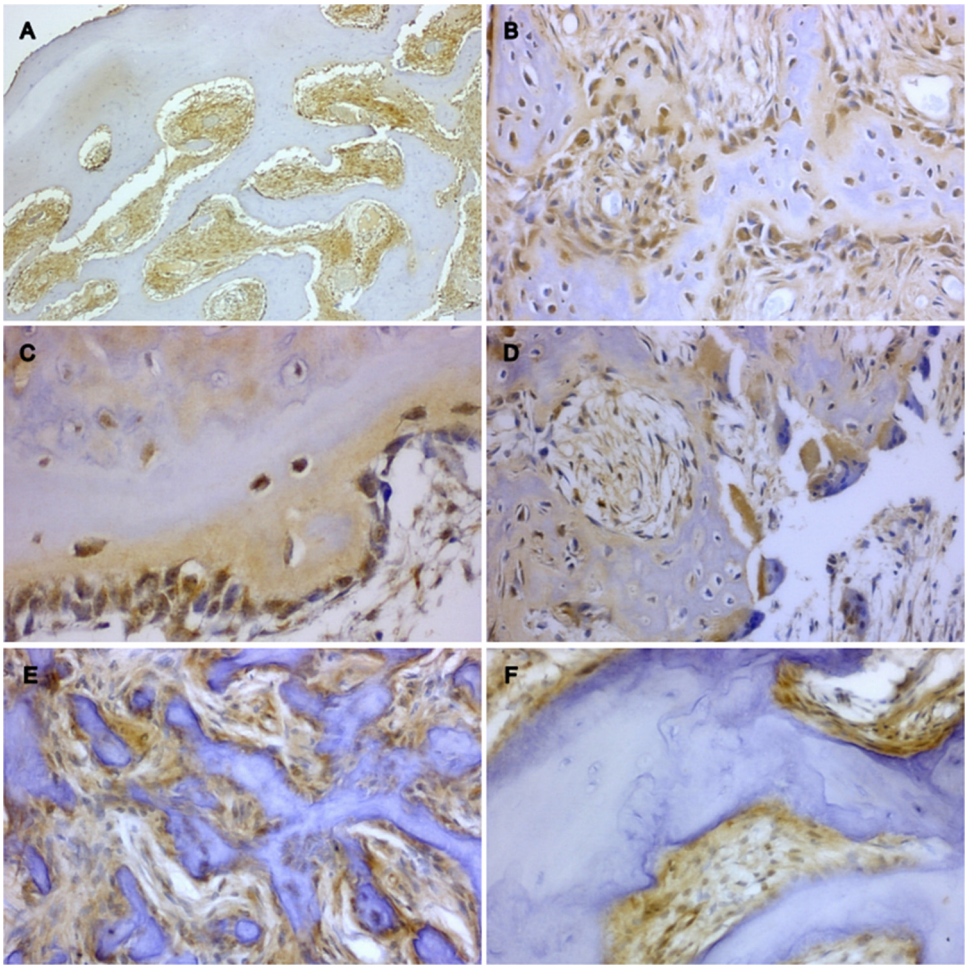

Fig. 2. Photomicrographs showing **OSTEONECTIN** expression in lesions samples. **A)** Fibrous Dysplasia: presence in fibrous matrix (X63); **B)** Fibrous Dysplasia: staining in osteoblasts and "young" osteocytes (X250); **C)** Fibrous Dysplasia: presence in osteoid, osteoblasts and osteocytes (X400); **D)** Fibrous Dysplasia: focal staining in osteoclasts (X250); **E)** Cemento-ossifying Fibroma: presence in peripheral cells (X250); **F)** Cemento-osseous Dysplasia: positivity in fibrous matrix (X250).

osteonectin (LF-37), human osteopontin (LF-166), human bone sialoprotein (LF-100) e bovine osteocalcin (LF-32), were obtained from Dr. Larry W. Fisher (Matrix Biochemistry Unit, National Institutes of Health - National Institute of Dental and Craniofacial Research, Bethesda, Maryland, USA). The antibody LF-32 can react with human osteocalcin and is able to identify it. Concentrations used were, respectively, 1:1000, 1:1200, 1:800, 1:1300, diluted in TRIS-HCL buffered. All of them were allowed to incubate, automatically, for 40 min. A pilot-study was performed to verify best dilutions for the antibodies, and to test both the tissue antigenic behavior and the LSAB staining method´s ability to work well with these antibodies.

After washing twice with TBST, the sections were incubated with a biotinylated swine-antimouse, rabbit, goat antibody (DAKO – LSAB Kit, DAKO Corporation, Carpinteria, MA, USA) for 30 min, rinsed as above, and then exposed to streptavidin-biotin peroxidase conjugate (DAKO – LSAB Kit, Peroxidase K0690) for 30 min. Antibody complexes were visualized after the addition of a buffered diaminobenzidine (Dako Liquid DAB plus K3468) substrate for 10 min. The reaction was stopped by immersion and rinsing twice in TBST. Sections were then counterstained with Mayer´s hematoxylin for 10 min, dehydrated with ascending alcohols, cleared with xylene and mounted in xylene-based Permount (Fisher Scientific, Fair Lawn, New Jersey/USA).

Negative controls comprised the use of TBST in place of the primary antibodies, and slides of normal bone tissue were included in all immunohistochemical incubations as positive controls. A qualitative analysis of the results was carried out using a light microscope. In each tissue slice, the immunoactivity was observed for a given protein in different locations: fibrous matrix, mineralized matrix, osteoid, osteoblasts, osteocytes and osteoclasts. Staining were considered positive, weakly positive or negative, with the designation "weakly positive" being reserved for cases where there was a lower intensity of impregnation of the chromogenic substance and/or heterogeneous labeling within the analyzed region, such as, for example, when expression in mineralized matrix was restricted to the periphery or incremental lines. For each labeling location, the N (total number of sample elements) varied, since not all cases had osteoclasts present or osteoid evident, for example. The cases were grouped according to the lesion (fibrous dysplasias, cemento-ossifying fibromas and cemento-bony dysplasias) and submitted to a statistical analysis. The Chi-square test or Fisher's test (whichever was applicable depending on the table pattern) was employed to find the association between desired variables. The result was considered statistically significant if $p < 0.05$. For statistical analysis, the software used was SPSS version 22.0 (IBM, Armonk, NY).

3. Results

Cases of Fibrous Dysplasia reacted positively for osteonectin in fibrous matrix (68,2% - observe Fig. 2A), osteoid (50% - Fig. 2B and C) and osteoblasts (77,3% - Fig. 2B and C). Osteocytes and osteoclasts were considered, in majority, slightly positives because of expression heterogeneity. "Young" osteocytes (recently embedded by the matrix or situated close to the osteoid trabecular surface in large, round lacunae)

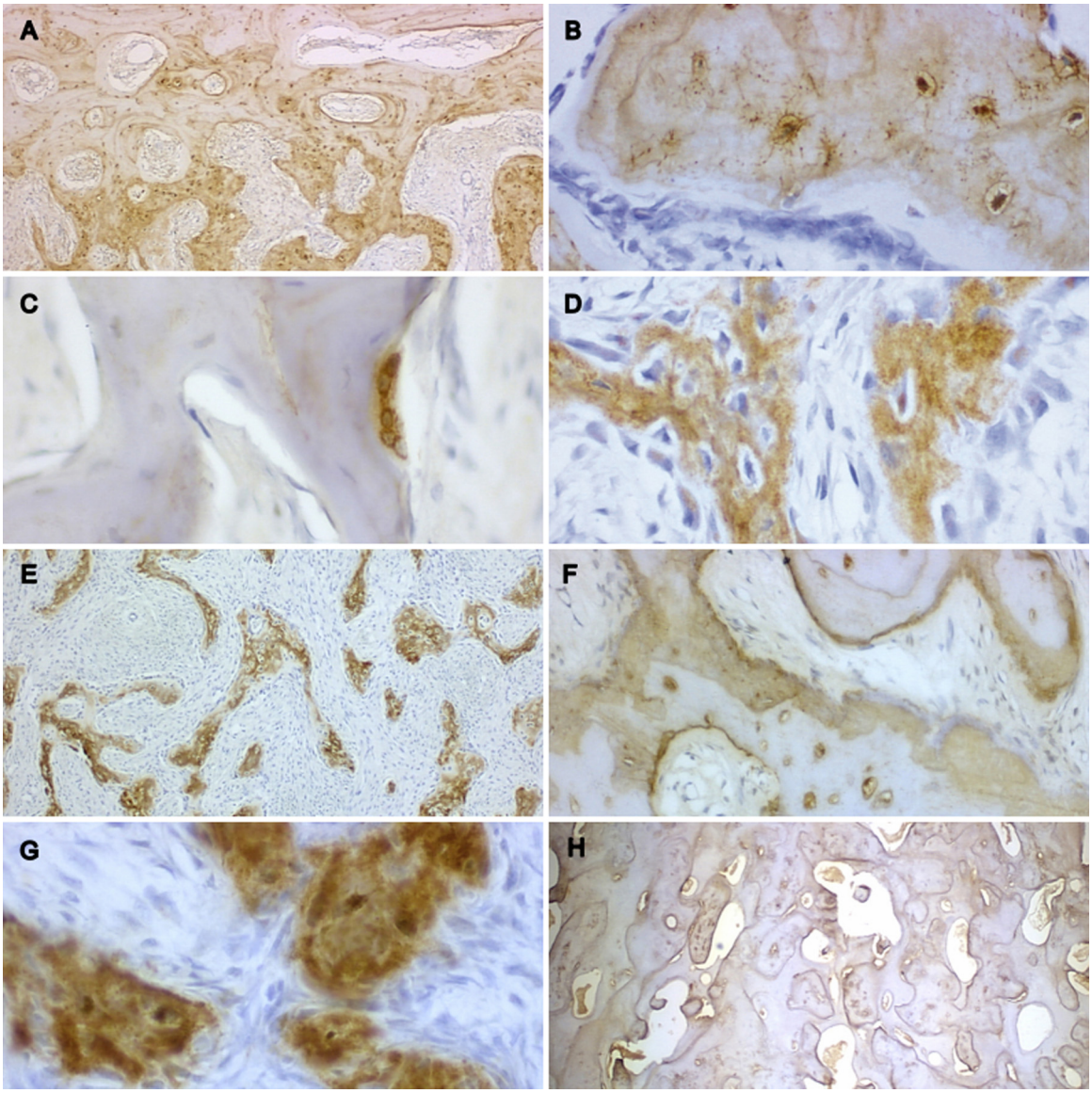

Fig. 3. Photomicrographs showing **OSTEOPONTIN** expression in lesions samples. **A)** Fibrous Dysplasia: staining profile in transition between bone cortical and dysplastic bone (X63); **B)** Fibrous Dysplasia: presence in osteocytes and their extensions (X400); **C)** Fibrous Dysplasia: perinuclear staining of osteoclasts (X400); **D)** Fibrous Dysplasia: presence in immature mineralized matrix and in osteoblasts (X400); **E)** Fibrous Dysplasia: intense positivity in immature mineralized matrix (X63); **F)** Fibrous Dysplasia: strong positivity in mineralized matrix periphery (X250); **G)** Cemento-ossifying Fibroma: intense positivity in mineralized matrix (X400); **H)** Cemento-osseous Dysplasia: heterogeneous staining in mineralized matrix (X63).

showed a stronger antibody binding than “old” osteocytes (smaller and centrally located in spindle-shaped lacunae), where marking by the osteonectin antibodies was weak or absent (Fig. 2B and C). For osteoclasts, the immunoreactivity was concentrated only at the portion of cytoplasm which faces the bone surface (Fig. 2D). Cases of Cemento-ossifying Fibroma showed immunostaining for osteonectin in fibrous matrix (75% - observe Fig. 2E), osteoid (31,3%), osteoblasts (81,3%) and the same heterogeneity of expression in osteocytes and osteoclasts. In cases of Cemento-osseous Dysplasia, osteonectin immunoreacted in fibrous matrix (75% - observe Fig. 2F), osteoid (80%) and osteoblasts (87,5%), keeping the same heterogeneous expression already verified in the other groups for osteocytes and osteoclasts.

In cases of Fibrous Dysplasia, there was strong immunoreactivity for osteopontin in mineralized matrix (95.5% - Fig. 3A), osteocytes (90.9% - Fig. 3B) and osteoclasts (100% in perinuclear region – Fig. 3C). Staining in osteoblasts was weakly positive in 86.4% of cases (Fig. 3D). Heavy and homogeneous staining for osteopontin was observed in immature mineralized tissue (see Fig. 3E); whereas a more densely mineralized tissue and with a tendency to lamelae formation also exhibited the protein, but concentrated in focal areas (Fig. 3F), mimicking the expression profile observed in normal bone tissue (present in upper side of Fig. 3A). In cases of Cemento-ossifying Fibroma, osteopontin was present, again, in mineralized matrix (87.5% of cases – Fig. 3G), as well as in osteocytes (76.9%) and osteoclasts (100%). At this time, however, osteoblasts were predominantly negative (81.2% - Fig. 3G). In cases of Cemento-osseous Dysplasia, osteopontin was strongly present in osteocytes (75%) and osteoclasts (100%). Its expression in mineralized matrix, however, was reduced to weakly positive in 100% of cases (Fig. 3H) and, in osteoblasts, varied among positive, negative and weakly positive, in almost the same proportion (31.3%, 31.3% e 37.4%, respectively). Fibrous matrix, negative for osteopontin in majority of cases until then, turns to exhibit a weakly positive staining in COD (56.3% - Fig. 3H).

In cases of Fibrous Dysplasia, expression of bone sialoprotein was strong in osteoid, osteoblasts and osteocytes (55%, 72.7% and 86.4% of cases, respectively - see Fig. 4A and B) and heterogeneous in osteoclasts

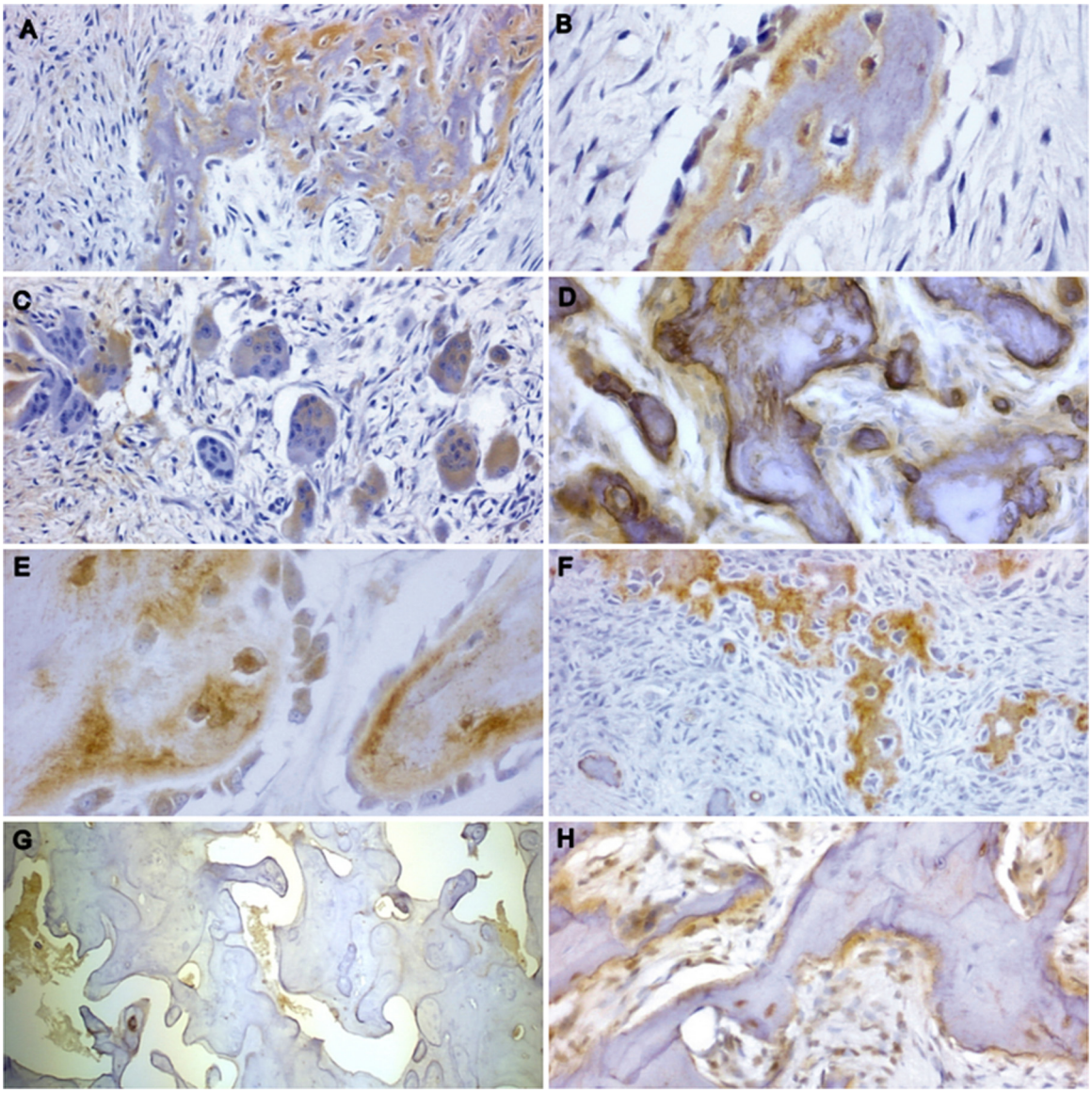

Fig. 4. Photomicrographs showing **BONE SIALOPROTEIN** expression in lesions samples. **A)** Fibrous Dysplasia: presence in mineralized matrix periphery (X250); **B)** Fibrous Dysplasia: staining in osteoblasts, osteocytes and trabeculae periphery (X400); **C)** Fibrous Dysplasia: heterogeneous presence in osteoclasts (X250); **D)** Cemento-ossifying Fibroma: positivity in globules periphery (X250); **E)** Cemento-ossifying Fibroma: presence in trabeculaes and osteoblasts (X400); **F)** Cemento-ossifying Fibroma: positivity in trabeculaes and negativity in globules (X250); **G)** Cemento-osseous Dysplasia: staining in fibrous matrix (X63); **H)** Cemento-osseous Dysplasia: presence in fibrous matrix and osteocytes (X250).

(57.9% - Fig. 4C), whereas mineralized matrix stained weakly (45.5% - Fig. 4A). In Cemento-ossifying Fibroma, bone sialoprotein exhibited intense immunoreaction in half of osteocytes (53.8%) and osteoclasts (50%). Mineralized matrix and osteoid were weakly positive in 50% and 43,7% of cases, respectively; whereas in majority of osteoblasts (68.7%), there was total absence of staining. An interesting finding was that BSP did not react with cementoid globules (as osteopontin did), neither with their peripheral cells; but was present in bone trabeculaes and their producing cells (as shown in Fig. 4D, E and, especially, F). For bone sialoprotein in Cemento-osseous Dysplasia, there was strong immunoreactivity in osteoid (80%), osteoblasts (62.5%) and osteocytes (56.3%); while osteoclasts appeared mildly stained (66.7%). The expression in fibrous matrix increased (43.7% - Fig. 4G and H), comparing to the other two groups of lesions.

In cases of Fibrous Dysplasia, osteocalcin was present in osteoblasts (63,7% - Fig. 5A and B) and osteocytes (63,7%), but also stained, although weakly, mineralized matrix (45,5% - Fig. 5A). In cases of Cemento-ossifying Fibroma, immunoreactivity for osteocalcin was poor in all areas (Fig. 5C), especially in osteoblasts, in which the positivity was only 12,5%. However, in osteocytes, the expression was positive in 46,2% (Fig. 5D). In Cemento-osseous Dysplasias, negativity showed the highest percentage values (Fig. 5E) for almost all regions. Weakly positive staining was observed for osteocalcin in osteoblasts (62,5%) and osteocytes (50%), sometimes strong for these ones (see Fig. 5F).

Although statistically significant differences have even been found in the distribution of the four proteins in the three studied lesions ($p < 0.05$), there was no polarization of results, that is, no concentration of staining frequency at the extremes (positivity or negativity), at the same time, in order to distinguish the lesions. It demonstrates that the use of these markers for differential diagnosis is still inviable.

4. Discussion

Comparing the imunostaining of osteonectin among all the three types of lesions, we can see it was present in fibrous matrix and absent

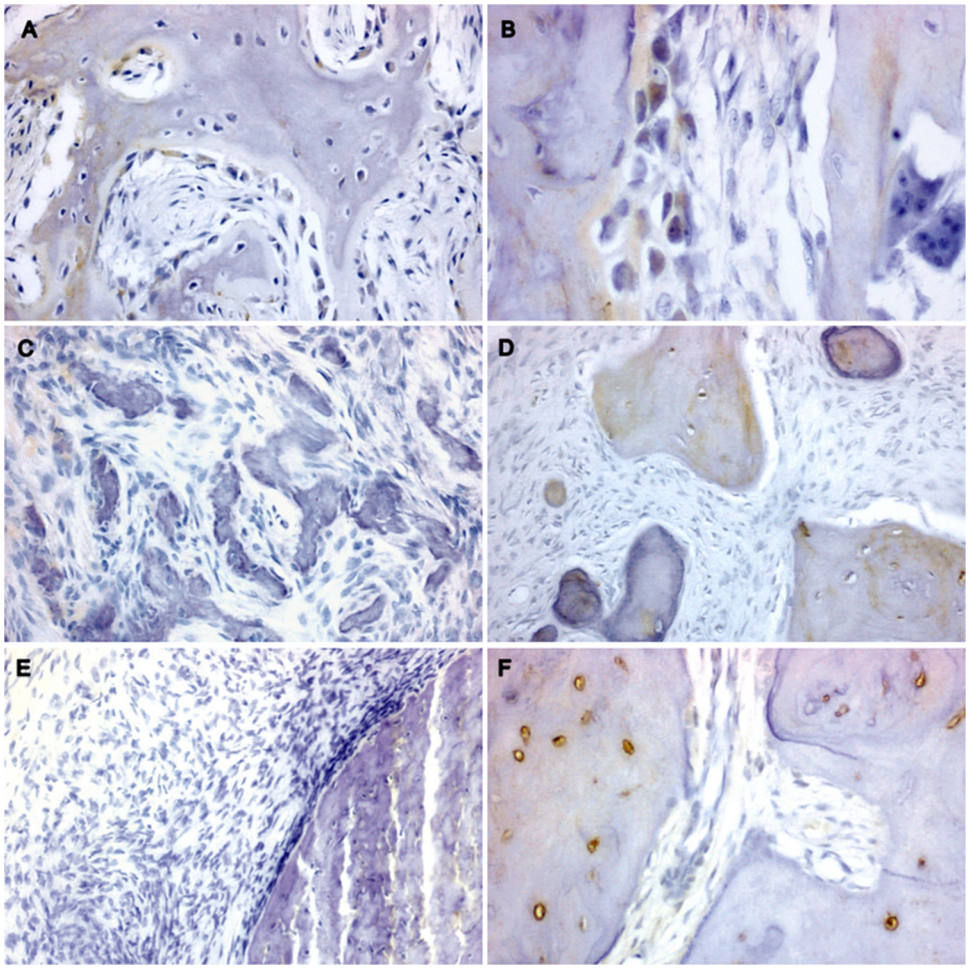

Fig. 5. Photomicrographs showing **OSTEOCALCIN** expression in lesions samples. **A)** Fibrous Dysplasia: presence in osteoblasts and focal areas of the mineralized matrix (X250); **B)** Fibrous Dysplasia: positivity in osteoblasts and negativity in osteoclasts (X400); **C)** Cemento-ossifying Fibroma: absence of staining (X250); **D)** Cemento-ossifying Fibroma: focal positivity in mineralized structures (X250); **E)** Cemento-osseous Dysplasia: absence of staining (X250); **F)** Cemento-osseous Dysplasia: intense immunoreaction in osteocytes (X250).

in mineralized one. Sakamoto et al., on the other hand, found positive staining in bone matrix, mainly in ossifying fibromas, comparatively to fibrous dysplasia [26]. Osteoid exhibited more evident positivity in Cemento-osseous Dysplasia, followed by Fibrous Dysplasia. Other authors have also found variable intensity of staining in osteoid, especially in cementoid globules periphery [11,13,14]. Osteoblasts were equally positive in the three groups of lesions, while osteocytes and osteoclasts kept heterogeneous imunoreactivity. Staining in osteoblasts, very emphasized in literature [14], exhibited a tendency of intensity loss as increases the distance from trabeculae. In fact, the fibrous matrix of Fibrous Dysplasias has osteoprogenitors cells of fibroblastic morphology, which can express premature markers of osteoblastic differentiation [27,28]. In osteocytes, the staining was very variable and its pattern was coincident with the one reported in the literature [11,14]. In cases of cemento-ossifying fibromas producers of cellular cementoid globules, the intensity of reaction was weaker, in agreement with the literature, that points out poor reactivity in the majority of the cementocytes. Osteoclasts, abundant in fibrous dysplasias and sporadic in cemento-ossifying fibromas and cemento-osseous dysplasias, were negative for osteonectin, with the exception of cytoplasmic portion which faces the reabsorbing bone surface.

Comparing the imunostaining of osteopontin among all the three types of lesions, we can see total absence of imunoreactivity in fibrous matrix. Mineralized matrix appeared positively stained in Fibrous Dysplasias and Cemento-ossifying Fibromas, while it was only weakly positive and limited to periphery and incremental lines in Cemento-osseous Dysplasias. Osteoid staining was almost invariably negative, which antagonizes with some authors [20,22,29], but could be attributed to differences in methodology. As it is present both in lamelar and woven bone [30] and appears in relatively early stages of osteoblastic differentiation [22], its expression is usually more intense in immature matrix, incremental lines and peripheral region of bone [20,22,29,31,32], as well as in cement matrix [33]. It is usually present in osteoblasts [14,15,19,20,22], especially if paraffinized slices are analyzed [22]. In our study, osteoblasts were weakly positive in Fibrous Dysplasias, negative in most cases of Cemento-ossifying Fibromas and very variable in Cemento-osseous Dysplasias. Spindle-shaped cells, disposed in the periphery of cementoid globules of cemento-ossifying fibromas, were negative, disaccording with studies that found positivity, even sporadic, in cells producing cementum, both tumoral and of root lining [19,25,33]. Osteocytes and osteoclasts were almost always positive. According to the literature, osteopontin can be expressed in the osteoclast´s surface of interface with bone matrix, represented by clear zone and ruffled border [15], but also in periphery and perinuclear region, as we could verify.

Comparing the imunostaining of bone sialoprotein among all the three types of lesions, we can see positivity in fibrous matrix almost only in Cemento-osseous Dysplasias. On the other hand, in mineralized matrix, some immunoreaction was observed in Fibrous Dysplasias. According to the literature, mineralized matrixes are positive for BSP [13,14,16,23,24], especially in paraffinized slices [20]. The imunostaining is even more intense in incremental lines [21,31] and in

areas of newly formed bone [13], mostly where deposition occurred quickly [23]. Literature also points out positivity for BSP in cellular and acellular cement [13,33], mostly in fast and early cementogenesis [13,24], but also emphasizes that there are significant differences inside tissues in different stages of maturation [10]. The protein was also present in osteoid, particularly in cases of Cemento-osseous Dysplasia and Fibrous Dysplasia. Although some authors had reported absence of expression [11,20], others show positivity in osteoid, even weak [26,33]. Osteoblasts represent the cellular type that expresses the highest levels of BSP [14,20,21,22,24], mostly when they exhibit cuboidal morphology and are depositing matrix [23]. In fact, cells that appear in bone trabeculae periphery show the profile of active osteoblasts and tend to present BSP, as we saw. Cementoblasts are considered a probable source of BSP [14,23,24,33], but spindle-shaped cells from periphery of cementoid structures, in our lesions, presented clear negativity [19,34]. Therefore, BSP seems to show a distinct profile between these two mineralized structures. Unfortunately, they can both coexist in the same lesion. Osteocytes were positive, especially in Fibrous Dysplasias. In the literature, staining in osteocytes seems controversial [21,22], as it can vary from positive [14] or weakly positive [23], to negative [20]. Osteoclasts expressed, but only in Fibrous Dysplasias and Cemento-ossifying Fibromas. Literature reports positivity [18,21].

Comparing the imunostaining of osteocalcin among the three types of lesions, we can see that absence of imunoreactivity is prevalent in all regions, except in fibrous matrix of some Cemento-osseous Dysplasias, in osteoblasts of Fibrous Dysplasias and in osteocytes, in a general way. Some authors pointed out positivity in mineralized matrix [11,35,36]. In cement, its expression varies according to the presence or not of embedded cells, and it can be positive [33], negative 35], or weekly positive [37]. As imunoreactivity for osteocalcin is usually more evident in lamelar or metabolically inactive bone [14,37,38], we can infer poor maturation in the sample lesions studied. Expression in osteoid, when positive, is weak [11,39]. In fact, most cases showed totally negative osteoid. Soft tissues are generally appointed as unable of reacting with osteocalcin [14]. In Cemento-osseous Dysplasias, however, weakly positive staining was seen. Osteoblasts, in a general way, are positive [14,35,39]. On the other hand, expression in cementoblasts is reported as sporadic [33], variable, and can be positive [37,38], especially in those responsible for cellular cementum formation [35], or negative [14], as in cells adjacent to acellular cementum [35]. In fact, in cases of fibrous dysplasias, most osteoblasts stained positively. In Cemento-osseous Dysplasias, expression varied from weakly positive to negative, while in most Cemento-ossifying Fibromas, where acellular cementoid globules are prevalent, peripheral spindle-shaped cells did not react, following a tendency already seen for OP and BSP. As osteocalcin is considered a well-differentiate osteoblast marker [14,22], it can be expected that osteocytes show intense staining. We observed, however, that these cells reacted strongly with osteocalcin in about half of the sample cases.

Actually, a heterogeneous cellular expression of these proteins can reflect maturational and functional differences [11,40], perfectly possible of occurrence in osteoblastic/cementoblastic population of the studied lesions, especially if we consider that they experience different evolutive phases, where blastic activity gradually overcomes the lytic one, and a progressive maturation occurs in mineralized component as well as in the producer cells [41–43].

5. Conclusions

The nature of mineralized structure, the cellular phenotype and the evolutive phase of maturation are much more responsible for the variations in expression of studied proteins than the type of lesion, which makes inviable the use of them with diagnostic purpose.

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Conflicts of interest

None.

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References

[1] F. Alawi, Benign fibro-osseous diseases of the maxillofacial bones. A review and differential diagnosis, Am. J. Clin. Pathol. 118 (2002) 50–70.
[2] D.S. MacDonald-Jankowski, Ossifying fibroma: systematic review, Dentomaxillofac. Radiol. 38 (2009) 495–513.
[3] K. Abramovitch, D.D. Rice, Benign fibro-osseous lesions of the jaws, Dent. Clin. N. Am. 60 (2016) 167–193.
[4] S.K. El-Mofty, Fibro-osseous lesions of the craniofacial skeleton: an update, Head Neck Pathol. 8 (2014) 432–444.
[5] R. Eversole, L. Su, S. ElMofty, Benign fibro-osseous lesions of the craniofacial complex. A review, Head Neck Pathol. 2 (2008) 177–202.
[6] C.A. Waldron, Fibro-osseous lesions of the jaws, J. Oral Maxillofac. Surg. 8 (1993) 828–835.
[7] A.K. El-Naggar, J.K.C. Chan, T. Takata, J.R. Grandis, P.J. Slootweg, The fourth edition of the head and neck World Health Organization blue book: editors' perspectives, Hum. Pathol. 66 (2017) 10–12.
[8] J.M. Wright, M. Vered, Update from the 4th edition of the World Health Organization classification of head and neck tumours: odontogenic and maxillofacial bone tumors, Head Neck Pathol. 1 (2017) 68–77.
[9] A.L. Boskey, Biomineralization: conflicts, challenges, and opportunities, J. Cell. Biochem. Suppl. 30 (31) (1998) 83–91.
[10] P.G. Robey, Vertebrate mineralized matrix proteins: structure and function, Connect. Tissue Res. 35 (1996) 131–136.
[11] J.R. Nefussi, G. Brami, D. Modrowski, M. Oboeuf, N. Forest, Sequential expression of bone matrix proteins during rat calvaria osteoblast differentiation and bone nodule formation in vitro, J. Histochem. Cytochem. 45 (1997) 493–503.
[12] E.A. Cowles, M.E. DeRome, G. Pastizzo, L.L. Brailey, G.A. Gronowicz, Mineralization and the expression of matrix proteins during in vivo bone development, Calcif. Tissue Int. 1 (1998) 74–82.
[13] M. Matsuura, Y. Herr, K.Y. Han, W.L. Lin, R.J. Genco, M.I. Cho, Immunohistochemical expression of extracellular matrix components of normal and healing periodontal tissues in the beagle dog, J. Periodontol. 66 (1995) 579–593.
[14] B. Sommer, M. Bickel, W. Hofstetter, A. Wetterwald, Expression of matrix proteins during the development of mineralized tissues, Bone 19 (1996) 371–780.
[15] C.M. Giachelli, S. Steitz, Osteopontin: a versatile regulator of inflammation and biomineralization, Matrix Biol. 19 (2000) 615–622.
[16] L.W. Fisher, D.A. Torchia, B. Fohr, M.F. Young, N.S. Fedarko, Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin, Biochem. Biophys. Res. Commun. 280 (2001) 460–465.
[17] A.L.J.J. Bronckers, M.C. Farach-Carson, E. Van Waeren, W.T. Butler, Immunolocalization of osteopontin, osteocalcin, and dentin sialoprotein during dental root formation and early cementogenesis in the rat, J. Bone Miner. 9 (1994) 833–841.
[18] A. Bellahcene, K. Bonjean, B. Fohr, N.S. Fedarko, F.A. Robey, M.F. Young, L.W. Fisher, V. Castronovo, Bone sialoprotein mediates human endothelial cell attachment and migration and promotes angiogenesis, Circ. Res. 86 (2000) 885–891.
[19] H. Arzate, M.A. Alvarez-Pérez, M.E. Aguilar-Mendoza, O. Alvarez-Fregoso, Human cementum tumor cells have different features from human osteoblastic cells in vitro, J. Periodontal Res. 33 (1998) 249–258.
[20] G.J. Pinero, M.C. Farach-Carson, R.E. Devoll, J.E. Aubin, J.C. Brunn, W.T. Butler, Bone matrix proteins in osteogenesis and remodelling in the neonatal rat mandible as studied by immunolocalization of osteopontin, bone sialoprotein, alpha 2HS-glycoprotein and alkaline phosphatase, Arch. Oral Biol. 40 (1995) 145–155.
[21] T. Kreis, R. Vale, Guidebook to the Extracellular Matrix, Anchor, and Adhesion Proteins, A Sambrook; Tooze Publication at Oxford University Press, New York, 1999.
[22] J.E. Aubin, Advances in the osteoblast lineage, Biochem. Cell Biol. 76 (1998) 899–910.
[23] B. Ganss, R.H. Kim, J. Sodek, Bone sialoprotein, Crit. Rev. Oral Biol. Med. 10 (1999) 79–98.
[24] J. Gao, A.L. Symons, H. Haase, P.M. Bartold, Should cementoblasts express alkaline phosphatase activity? Preliminary study of rat cementoblasts in vitro, J. Periodontol. 70 (1999) 951–959.
[25] B.M. Eley, S.W. Cox, Advances in periodontal diagnosis. 10. Potential markers of bone resorption, Br. Dent. J. 10 (1998) 489–492.
[26] A. Sakamoto, Y. Oda, Y. Iwamoto, M. Tsuneyoshi, A comparative study of fibrous dysplasia and osteofibrous dysplasia with regard to expressions of c-fos and c-jun products and bone matrix proteins: a clinicopathologic review and

immunohistochemical study of c-fos, c-jun, type I collagen, osteonectin, osteopontin, and osteocalcin, Hum. Pathol. 30 (1999) 1418–1426.
[27] M.M. Cohen Jr, R.E. Howell, Etiology of fibrous dysplasia and McCune-Albright syndrome, Int. J. Oral Maxillofac. Surg. 28 (1999) 366–371.
[28] P.J. Marie, C. de Pollak, P. Chanson, A. Lomri, Increased proliferation of osteoblastic cells expressing the activating Gs alpha mutation in monostotic and polyostotic fibrous dysplasia, Am. J. Pathol. 150 (March) (1997) 1059–1069.
[29] Y. Sasano, J.X. Zhu, S. Kamakura, S. Kusunoki, I. Mizoguchi, M. Kagayama, Expression of major bone extracellular matrix proteins during embryonic osteogenesis in rat mandibles, Anat. Embryol. (Berl.) 202 (2000) 31–37.
[30] J.P. Gorski, Is all bone the same? Distinctive distributions and properties of noncollagenous matrix proteins in lamellar vs. woven bone imply the existence of different underlying osteogenic mechanisms, Crit. Rev. Oral Biol. Med. 9 (1998) 201–223.
[31] A. Nanci, Content and distribution of noncollagenous matrix proteins in bone and cementum: relationship to speed of formation and collagen packing density, J. Struct. Biol. 126 (1999) 256–269.
[32] J. Sodek, B. Ganss, M.D. McKee, Osteopontin, Crit. Rev. Oral Biol. Med. 11 (2000) 279–303.
[33] R.L. MacNeil, J.A. D'Errico, H. Ouyang, J. Berry, C. Strayhorn, M.J. Somerman, Isolation of murine cementoblasts: unique cells or uniquely-positioned osteoblasts? Eur. J. Oral Sci. 106 (1998) 350–356.
[34] J.A. D'Errico, R.L. MacNeil, T. Takata, J. Berry, C. Strayhorn, M.J. Somerman, Expression of bone associated markers by tooth root lining cells, in situ and in vitro, Bone 20 (1997) 117–126.
[35] M. Kagayama, H.C. Li, J. Zhu, Y. Sasano, Y. Hatakeyama, I. Mizoguchi, Expression of osteocalcin in cementoblasts forming acellular cementum, J. Periodontal Res. 32 (1997) 273–278.
[36] S. Toyosawa, M. Yuki, M. Kishino, Y. Ogawa, T. Ueda, S. Murakami, E. Konishi, S. Iida, M. Kogo, T. Komori, Y. Tomita, Ossifying fibroma vs fibrous dysplasia of the jaw: molecular and immunological characterization, Mod. Pathol. 20 (2007) 389–396.
[37] S. Ivanovski, H. Li, T. Daley, P.M. Bartold, An immunohistochemical study of matrix molecules associated with barrier membrane-mediated periodontal wound healing, J. Periodontol Res. 35 (2000) 115–126.
[38] H. Zhou, P. Choong, R. McCarthy, S.T. Chou, T.J. Martin, K.W. Ng, In situ hybridization to show sequential expression of osteoblast gene markers during bone formation in vivo, J. Bone Miner. Res. 9 (1994) 1489–1499.
[39] D. Tenorio, A. Cruchley, F.J. Hughes, Immunocytochemical investigation of the rat cementoblast phenotype, J. Periodontal Res. 28 (1993) 411–419.
[40] S.S. Barros, E.J. Gimeno, Cell differentiation and bone protein synthesis in the lungs of sheep with spontaneous calcinosis, J. Comp. Pathol. 123 (2000) 270–277.
[41] D.S. MacDonald-Jankowski, Cemento-ossifying fibromas in the jaws of Hong Kong Chinese, Dentomaxillofac. Radiol. 27 (1998) 298–304.
[42] J.M. Wright, Reactive, dysplastic and neoplastic conditions of periodontal ligament origin, Periodontology 21 (1999) 7–15.
[43] T. Kawai, H. Hiranuma, M. Kishino, A. Jikko, M. Sakuda, Cemento-osseous dysplasia of the jaws in 54 Japanese patients: a radiographic study, Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 87 (1999) 107–114.