UNIVERSIDADE TIRADENTES
CURSO DE ODONTOLOGIA

UM NOVO E PROMISSOR PRODUTO NATURAL PARA CAPEAMENTO PULPAR DIRETO BASEADO EM OTÓLITOS DE CYNOSCION ACOUPA: ESTUDO EXPERIMENTAL EM CÃES.

Trabalho de Conclusão de Curso apresentado à Coordenação do Curso de Odontologia da Universidade Tiradentes como parte dos requisitos para obtenção do grau de bacharel em Odontologia.

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– 2ª examinador

UNIT

“Na batalha da vida só vencem os fortes, e um homem forte, sempre determina seu destino!”
(Autor Desconhecido)

AGRADECIMENTOS

Agradeço...

A Deus, pela vida, pela paz, pela família, pelos amigos, pela coragem e pela força que tem me dado todos os dias dessa jornada.

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As pessoas e amigos que estiveram ao meu lado antes e durante esta conquista.

Obrigado a todos

Resumo

O Capeamento pulpar direto é um procedimento que consiste na adequada proteção do tecido pulpar exposto ao meio bucal, a fim de selar o local de exposição contra infiltração bacteriana e preservar a vitalidade pulpar, bem como sua função nutricional, sensorial, protetora e formadora de barreira de tecido mineralizados. Otólitos são concreções calcárias encontrados no ouvido interno dos peixes ósseos, constituídos por uma grande variedade de compostos inorgânicos e proteínas colagenosas de alto peso molecular, aparentemente relacionadas ao processo de calcificação de tecidos duros humanos. Este estudo avaliou a resposta da polpa dental de cães ao capeamento com otólitos e hidróxido de cálcio. Para tanto, dois cães foram anestesiados e, após isolamento absoluto com dique de borracha, a polpa dentária dos incisivos foi experimentalmente exposta de forma padronizada e as cavidades foram seladas com cimento de ionômero de vidro. Posteriormente, os 24 dentes foram divididos em três grupos, de acordo com o material capeador empregado: preparação otólitos (OTL), hidróxido de cálcio (HC) e sem material de proteção no terceiro grupo (CTR). Vinte e um e 30 dias após o experimento, os animais foram anestesiados, os dentes foram extraídos e submetidos à tomografia computadorizada de feixe cônico, radiografia convencional e preparados para análise histomorfológica. Os dados foram comparados por meio do teste de Fischer (α=5%). Os otólitos mostraram biocompatibilidade com o tecido pulpar *in vivo* e a resposta do tecido pulpar em OTL foi semelhante àquela evidenciada em HC. Além disso, uma barreira mineralizada com a aparência dentinóide foi observada em 100% do grupo HC, enquanto que 83,3% do grupo OTL formaram uma barreira de aspecto osteóide selando a área de exposição. Não houve formação de barreira no CTR, fosse em 21 ou em 30 dias. Não houve diferença estatisticamente significante entre OTL e HC ($p < 0,05$) nos períodos estudados. Conclui-se que o capeamento pulpar direto com otólitos é capaz de preservar a vitalidade pulpar, estimulando a formação de barreira de tecido mineralizado e induzir a resposta pulpar reparadora e, portanto, representa um promissor biomaterial para capeamento pulpar direto a ser usado na odontologia.

PALAVRAS-CHAVES: Otólitos; Capeamento pulpar direto; materiais biocompatíveis, hidróxido de cálcio; cavidade da polpa dentária.

A novel promising natural product for direct dental pulp capping based on Cynoscion acoupa otoliths: experimental study in canine model.

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A novel promising natural product for direct dental pulp capping based on Cynoscion acoupa otoliths: experimental study in canine model.

ABSTRACT:

Direct pulp capping is a procedure that comprises adequate protection of the pulp tissue exposed to the oral environment, in order to seal this spot against bacterial leakage, and preserve the pulp vitality as well as its nutritional, sensory, protection and formation of tissue barrier mineralized functions. Otoliths are calcareous concrescences found in the inner ear of bony fishes, constituted of a wide range of inorganic compounds and high-molecular weight collagenous proteins apparently related to the human hard tissues calcification process. This study evaluated the response of the dental pulps of dog teeth to capping with otolith, calcium hydroxide or sealed glass ionomer cement. Therefore, two dogs were anesthetized and, after placement of a rubber dam, the dental pulps the incisors were experimentally exposed. Subsequently, the teeth were assigned into three groups, according to the capping material employed: otoliths preparation (OTL) and calcium hydroxide (HC); no protective material was used in the third group (CTR). The cavities were then sealed with glass ionomer cement. Twenty-one and 30 days after the experiment, the animals were anesthetized, the teeth were extracted and subjected to cone-beam computerized tomography, X-rayed and prepared for histomorphological analysis. The data were compared by Fisher test (α=5%). The otoliths showed biocompatibility with the pulp tissue *in vivo* and the response of pulp tissue in OTL was similar to those produced in HC. Besides, mineralized barrier with dentin-like appearance was observed in 100% HC, whereas 83.3% of OTL formed an osteoid barrier sealing the spot area. No hard tissue formation was seen in CTR either in 21 or in 30 days. There was no statistically significant difference between OTL and HC ($p<0.05$). We concluded that the otoliths stimulates the formation of mineralized tissue barrier and inducing the reparative pulp response, and hence work as a promising pulp capping material.

DESCRIPTORS: Otoliths. Dental pulp capping. Biocompatible Materials. Calcium hydroxide. Dental pulp cavity.

1. Introduction

Recently, many studies have been performed in attempt to improve bone regeneration by using acellular or cellular implantable materials[11]. Biominerals are an alternative class of biomaterials able to induce or improve bone formation in substitution to the autogenic grafts when applied into large bone defects[2, 25]. They consist of an inorganic phase (or phases) (usually simple salts or oxides) and a range of biomolecules that are often proteins, but may be carbohydrates, lipids or low-molecular-weight (<1 kDa) molecules such as polyamines. These biominerals are widely formed by bacteria, single-celled protists, plants, invertebrates and vertebrates including humankind[22].

The use of natural products with biomineral physical-chemical features has been target of a wide range of investigations in the bioengineering field, particularly aining at improving the biomineralization process based on calcium carbonate[21]. The *Cynoscion acoupa* (Weakfish, specie of the *Sciaenidae* family) is an important world fishing resource, presenting about 70 genders and 270 species widely found in Atlantic, Pacific and Indic oceans. It is found mainly in marine and estuary coast waters. In South America, it is concentrated in tropical and subtropical waters from the Brazilian coast, along all the coastal zone[15].

Among the hard structures of the *Cynoscion acoupa*, it is possible to highlight the otoliths, a calcarian compound observed in the inner ear of bony fishes. Otoliths are crystalline structures, comprised primarily of calcium carbonate, located in the inner ear of bony fishes, which function as balance organa[5,9]. Studies have indicated that otoliths are rich in calcium carbonate, metallic elements (Sr, Ba, Mg, Cd, Co, Cu, Zn, Na, K) and non-metallic compounds (Si, P, S, B)[4], as well as in a high molecular weight collagenous protein called otolin-1 and otoliths protein matrix-1[18,19,20,27], added to carbohydrates and lipids[6]. Therefore, despite otolith applications strayed so far across taxonomic and methodological boundaries, due to its chemical composition it is possible to suppose that this material might work as a biomineral.

Otolin-1 is a high molecular weight protein whose C-terminal domains present homology with type VIII and X collagen ones[18,20], proteins closely related to the mineralization process of non-calcified matrices[26]. It has been suggested that this otoliths protein represent a site of calcium carbonate ($CaCO_3$) deposition[18,24] and hence facilitate the calcification process, just as occurs in bone and dentin by using calcium phosphate ($CaPO_4$)[2,18].

Due to the high incidence and prevalence of caries disease, the damage of the dental organ for carious processes may sometimes lead to dental pulp exposition, as a result of the carie-related desmineralization dynamics itself or during the dentistry operative procedures[17]. It is well-established that depending on the inflammation grade of the dental pulp, it is possible that this specialized connective tissue recovers in response to conservative procedures, by means of the formation of mineralized barrier to seal the exposition[28].

Therefore, the goal of this study was to investigate the role played by otoliths of *Cynoscion acoupa* as biomineral adjuvant in biological assays of mineralized barrier formation in experimentally exposed dental pulps in canine model.

2. Material and methods

2.1 – Obtaining of the otoliths preparation:

The material in this study was prepared with 1 g powder of otolith of *Cynoscion acoupa* with particle size 60 mesh and addiction 0,25 g of hydrolyzed collegen, diluted in distilled water. The final product was packaged in dishes Petri and sterilized in UV rays (25 min). Subsequently, 1.0 g of the otoliths was diluted in 100 mL of distillated water, and the pH of the compound was assessed by using phmetro Digimed® (São Paulo, SP, Brazil) according to the manufacturer instructions.

2.2 - Operative procedures

The superior and inferior incisors of two mongrel dogs (male gender; age: 12-36 months; weight: 10-13 Kg), totaling 24 teeth, were selected for treatment. The specimens were identified to 1 until 12 for each animal. Therefore, the animals were pre-anesthetized with acepromazine (0.1 mg/Kg) (Acepran®, Univet Indústria Veterinária S.A., Campo Grande, MS, Brazil) and tramadol (2.0 mg/Kg) (Ultracet®, JANSSEN-CILAG Famarcêutica Ltda., São José dos Campos, SP, Brazil). The anesthesia was obtained by using propofol (5.0 mg/kg EV) (Diprivan®, Astra Zeneca do Brasil Ltda., Cotia, SP, Brazil) and maintained with isoflurano® (Instituto BioChimico Indústria Farmacêutica Ltda., Rio de Janeiro, RJ, Brazil) solution diluted in oxygen 100%. Local complementary infiltrate anesthesia was also performed with 1.0 mL lidocaine hydrochloride (Xylestesin®, Cristália Produtos Químicos Farmacêuticos Ltda., Itapira, SP, Brazil).

Crowns were cleaned, a rubber dam was placed and antisepsis with 2% chlorhexidine was performed. Coronal access on the buccal surface (Class V) was performed with high-speed nº1012 spherical diamond burs with cooling (Vortex®

Indústria e Comércio de Ferramentas Diamantadas Ltda, São Paulo, SP, Brazil), under refrigeration. After new antisepsis, the pulp chamber was exposed, and the homeostasis and dentine chip removal was obtained by irrigation of the pulp with saline solution. The dental pulp health status after the operative procedures was evaluated by visual (hot red color), tactile (firm texture) analysis, and spontaneous bleeding.

The pulp tissue was covered, aided for syringe Centrix® (Copyright © DFL Indústria e Comércio S.A., Rio de Janeiro, RJ, Brazil), with either otoliths preparation (OTL, 06 teeth/animal – specimens 4, 5, 6, 7, 8 e 9) or calcium hydroxide p.a. paste (Asfer Indústria Química Ltda., São Caetano do Sul, SP, Brazil) mixed with distillated water (HC, 03 teeth/animal – specimens 1, 2 e 3). In six teeth (03 teeth/animal – specimens 10, 11 e 12), the pulps remained uncovered, so that they might work as negative control (CTR). In the three groups, the teeth were restored with glass ionomer cement (Bioglass R®, Biodinâmica Química e Farmacêutica Ltda., Ibiporã, PR, Brazil), without previous acid conditioning. All the commercial products employed in this study were manipulated according to the manufacturer instructions. Twenty-one and 30 days after the operative procedures, the teeth were extracted and fixed in buffered formalin (pH 7.4). Euthanasia of the animals was not required and they were sent to adoption. The animals received post-operative medication with meloxican (Movatec®, Boehringer Ingelheim do Brasil Química e Farmacêutica LTDA., Itapecerica da Serra, SP, Brazil) (0.1 mg/Kg, 24/24 h) and tramadol (2.0 mg/Kg, 8/8 h), for seven days.

2.3 - Imaginological study

The radiographic study was performed in a Dabi Atlante (Ribeirão Preto, SP, Brazil) Apparatus (70kVp, 8 mA), focal distance of 20 cm, and exposition time corresponding to 0.5 s, by using periapical films Ultra-speed D® (Eastman kodak Company, Rochester, New York, USA) and positioner Rinn® (Rinn Corporations, Elgin, Illinois, USA).

The computed tomographic study was performed with Dento-Maxillofacial Limited Cone-beam Super High Resolution CT (i-CAT Imaging Sciences Internacional®, USA). The size of imaging volume was a cylinder with diameter 40 X height 30 mm at the X-ray rotational center. Images were taken under the exposure condition of 80 kV (X-ray tube voltage) and 4 mA (X-ray tube electric current), which were the standard parameters and can be changed for different subjects. A small cone-shaped X-ray beam irradiated the image intensifier with a CCD camera for approximately 40 s while the C-arm made one 3608 rotation. The panoramic reconstructions were carried out using software Xoran.

The imaginological analysis was performed considering the parameters absence (-) ou presence (+) of image consistent with mineralized barrier.

2.4 - Histological processing and morphological analysis

The teeth were submitted to demineralization procedures in EDTA (Ethylenediamine tetraacetic acid) for 96 h. The teeth were then washed in running water for 24 h, dehydrated by increasing concentrations of ethyl alcohol, cleared in xylol, and embedded in paraffin blocks. Serial 6-µm wide longitudinal sections were stained with hematoxylin and eosin and analyzed in light microscope.

During the histopathological analysis, and the following parameters were evaluated and graded according to a numerical scale: a) intensity of inflammatory infiltrate: grade 0, absent, grade 1, mild; grade 2, moderate; grade 3, severe; b) type of inflammatory infiltrate: acute, subacute or chronic; c) mineralized barrier: grade 0, absent; grade 1, presence of discontinuous barrier; grade 2, presence of continuous barrier. The histopathological analysis was performed by one examiner who was blinded to the group being evaluated.

2.5 - Statistical analysis

The presence and the type of mineralized barrier were compared among the groups by using the Exact Fisher´s test. The analysis of the inflammatory infiltrate was carried out by using Kruskal Wallis test. The differences were considered significant when p values were less than 0.05.

3. Results

The pH of the otoliths preparation was 8.77, and the biomaterial proved to be radiopaque.

According to the radiographic evaluation (figure 1a/b/c), in 21 days it was observed the presence of mineralized barrier in 100% of the specimens of HC and OTL, but not in CTR. In the CT analysis (figures 2 and 4), the barrier was visualized in 33.3% of CTR, and 100% of HC and OTL. In 30 days, the mineralized barrier was seen in 100% of HC and, 83.3% of OTL in both CT and radiographic studies (figures 3 and 4). However, although a radiopaque image compatible with hard tissue radiographically apparent in 66.6% of CTR, this finding was not confirmed by CT scans (table 1).

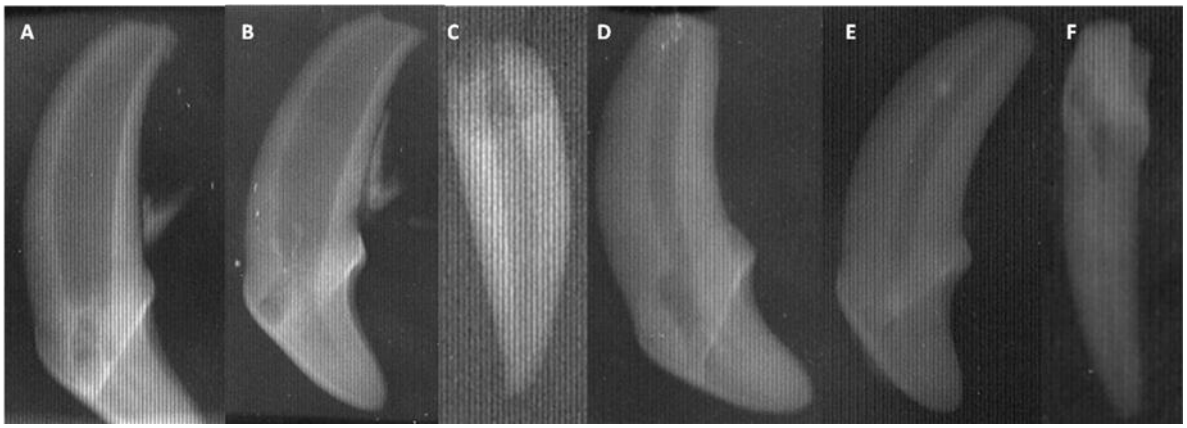

Figure 1. Radiographic appearance of the mineralized barrier in HC (A and D) and OTL (B and E) 21 and 30 days after the operative procedures, respectively. Observe the absence of barrier in CTR (C and F).

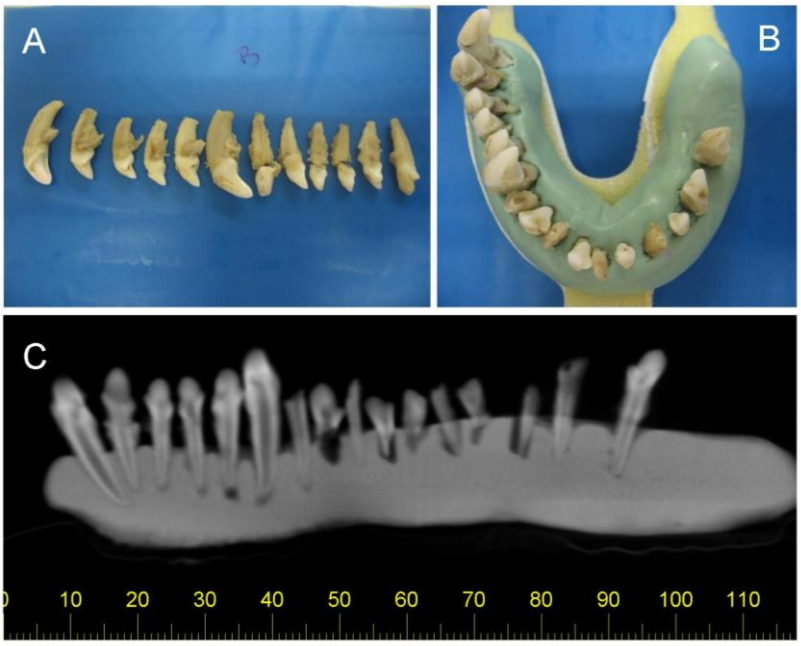

Figure 2. Teeth extracted 21 days after the operative procedures (A), and mounted for the CT scanning (B). Panoramic view of the cone beam CT scan (C).

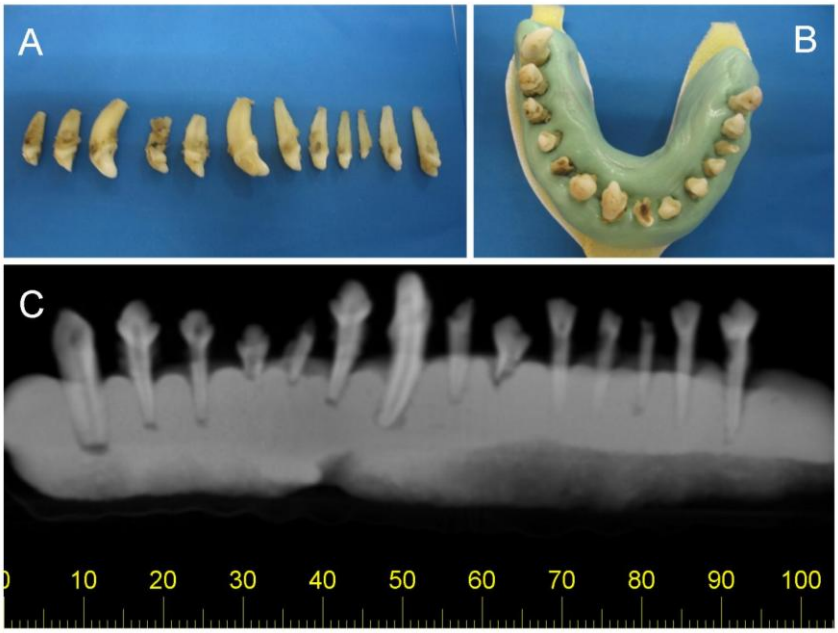

Figure 3. Teeth extracted 30 days after the operative procedures (A), and mounted for the CT scanning (B). Panoramic view of the cone beam CT scan (C).

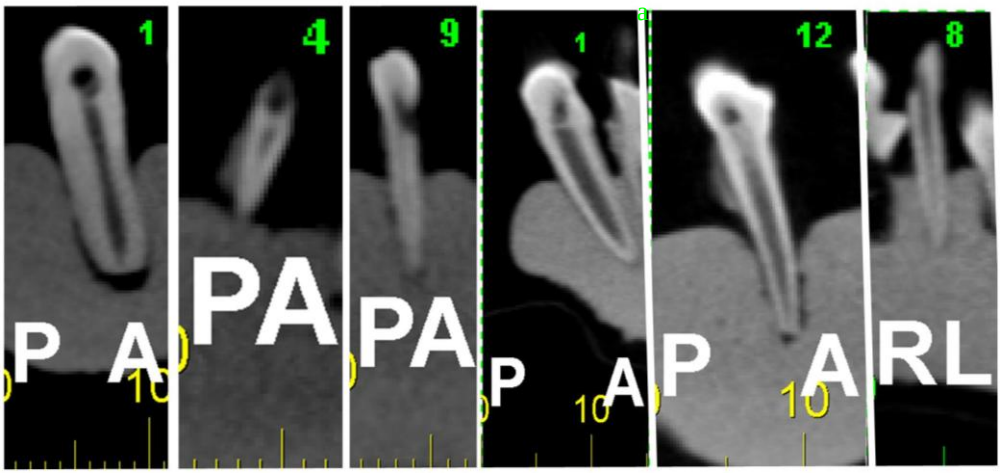

Figure 4. Details of the tomographic images of the mineralized barrier in HC (1) and OTL (4), which is not seen in CTR (9) 21 days after the operative procedures. In 30 days, it is possible to evidence well-formed barrier in HC (1a) and OTL (12), and lack of such structure in CTR (8)

Table 1. Frequency of the presence and absence of mineralized barrier observed in the radiographic and tomographic analysis.

Time	Mineralized barrier	Groups					
		CTR n (%)		HC n (%)		OTL n (%)	
		RA	TA	RA	TA	RA	TA
21days	Present	0 (0)	1 (33.3)	3 (100)	3 (100)	6 (100)	6 (100)
	Absent	3 (100)	2 (66.6)	0 (0)	0 (0)	0 (0)	0 (0)
	Total	3 (100)	3 (100)	3 (100)	3 (100)	6 (100)	6 (100)
30 days	Present	2 (66.6)	0 (0)	3 (100)	3 (100)	5 (83.3)	5 (83.3)
	Absent	1 (33.3)	3 (100)	0 (0)	0 (0)	1 (16.6)	1 (16.6)
	Total	3 (100)	3 (100)	3 (100)	3 (100)	6 (100)	6 (100)

RA: Radiographic analysis; TA: Tomographic analysis.

After hemi-section of the decalcified teeth, it was possible to verify, macroscopically, the presence of hard structure in bridge, consistent with dental mineralized barrier in some specimens of HC and OTL (figure 5), strongly suggesting the success of the experiment.

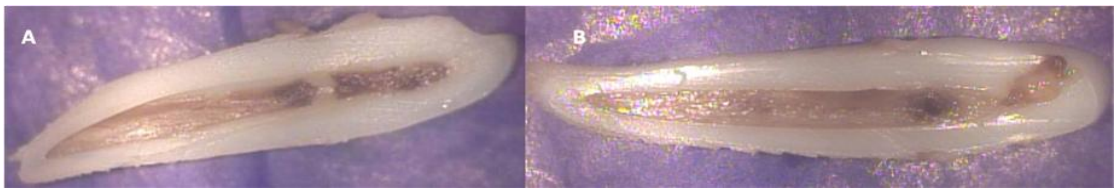

Figure 5. Macroscopic evidence of hard structures in bridge compatible with dental mineralized barrier occluding the pulp exposition zone in tooth capped with HC (A) and OTL (B) 30 days after the operative procedures

Histologically, it was observed the formation of mineralized barrier in all the specimens of HC and 66.6% of OTL 21 days after the operative procedures. In 30 days, all teeth of HC and OTL exhibited mineralized barrier formation. No sample of CTR showed hard tissue formation either in 21 or in 30 days (table 2). There was no statistically significant difference among HC and OTL regarding to the formation and type of deposited barrier ($p>0.05$).

Furthermore, the histological appearance of the barrier was different in those two groups. In HC, it was composed of a homogeneous eosinophilic tissue, with variable thickness, showing no cells within the matrix, mimicking a dentin-like morphology. In the periphery of the hard tissue, ovoid cells disposed in a pseudo-stratified arrangement, resembling odontoblasts, were observed rimming the newly-formed hard tissue. In OTL, on the other hand, there was deposition of thin irregular

matrix showing osteoid appearance, apparently surrounding grossly granular amorphous basophilic nuclei compatible with dystrophic calcification (figures 6 and 7).

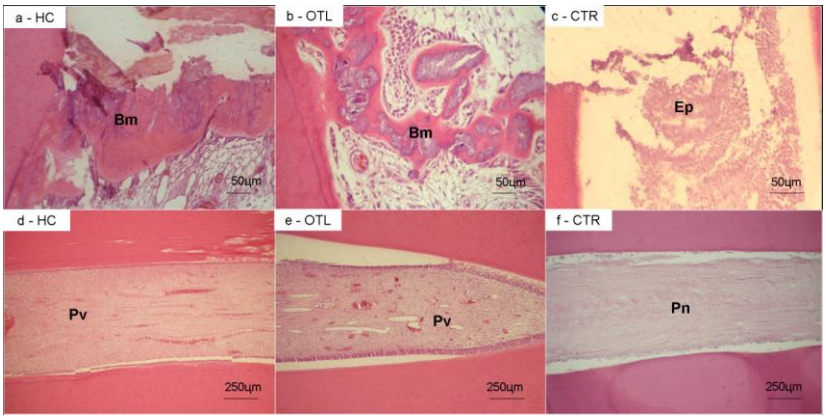

Figure 6. Histological sections stained in HE (21 days). Formation of mineralized barrier (Bm) with dentin-like appearance in HC (a-HC) and resembling osteoid in OTL (a-OTL). No occlusive barrier is seen in CTR in the exposition zone (Ep) (c-CTR). The remaining pulp (middle third) presented conspicuous network of hyperemic capillary blood vessels (Pv) in HC (d-HC) and OTL (e-OTL), whereas in CTR (f-CTR) there was morphological signs of coagulative necrosis (Pn).

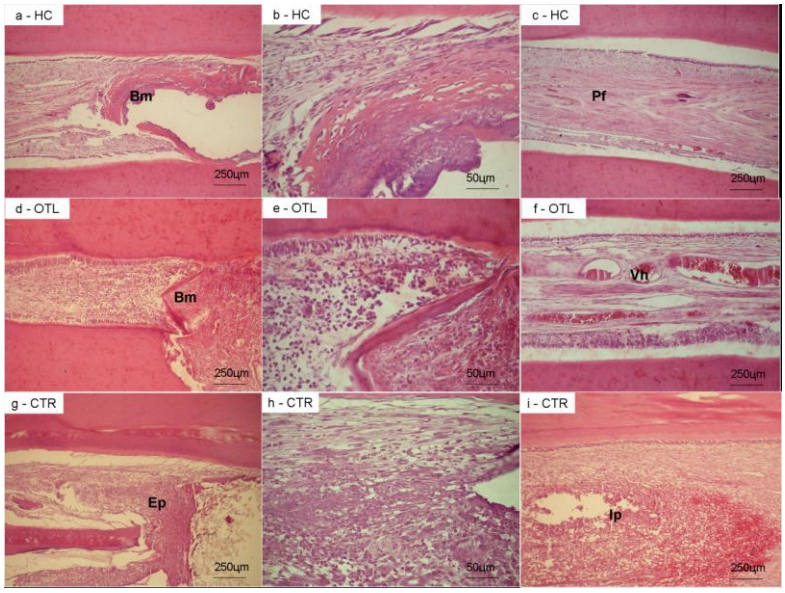

Figure 7. Histological sections stained in HE (30 days). Formation of mineralized barrier (Bm) in HC (a-HC) and OTL (d-OTL), whereas no barrier was seen in the exposition zone (Ep) in CTR (g-CTR). Adjacent to the exposition zone, there was scarce chronic infiltrate in HC (b-HC), but moderate in OTL (e-OTL). In CTR (h-CTR), there was intense acute infiltrate. In the remaining pulp middle third, it was observed fibrosis (Pf) in HC (c-HC), high content of hyperemic blood vessels (Vh) in OTL (f-OTL), and prominent acute infiltration (Ip), sometimes presenting microabscesses, in CTR (i-CTR).

Table 2. Frequency of the presence and type of mineralized barrier observed in the histological analysis.

Time	Mineralized barrier	Groups		
		CTR n (%)	HC n (%)	OTL n (%)
21days	Continuous	0 (0)	2 (66.6)	2 (33.3)
	Discontinuous	0 (0)	1 (33.3)	2 (33.3)
	Absent	3 (100)	0 (0)	2 (33.3)
	Total	3 (100)	3 (100)	6 (100)
30 days	Continuous	0 (0)	2 (66.6)	3 (50)
	Discontinuous	0 (0)	1 (33.3)	3 (50)
	Absent	3 (100)	0 (0)	0 (0)
	Total	3 (100)	3 (100)	6 (100)

The analysis of the inflammatory infiltrate showed that, in general, the severity of the inflammation tended to be more expressive in the cervical zone, reducing substantially in the middle and apical thirds. In 21 days, the pattern of inflammation was similar among the groups. In the cervical pulp thirds, the inflammatory severity ranged from moderate to intense, whereas in the middle and apical thirds, it varied between mild and absent. In 30 days, there was a slight decrease in the severity of the inflammation in the cervical pulp third in HC, although it appeared to increase in CTR and OTL. In the middle and apical pulp thirds the inflammatory profile remained mild or absent in HC and OTL. However, in CTR, necrosis was verified in two samples of 21 days, and in one sample of 30 days (table 3). There was no significant difference among the groups concerning the inflammatory infiltrate ($p>0.05$).

Table 3. Semiquantitative analysis of the inflammatory infiltrate observed in the dental pulp of the teeth used in the experiment.

Time	Pulp third	Groups											
		CTR			HC			OTL					
21days	Cervical	2*	n	2	3*	3*	2	2	2	2*	2	3*	2
	Middle	n	n	2	3	2	0	1	1	0	0	2	1
	Apical	n	n	2	0	0	0	0	0	0	0	0	0
30 days	Cervical	2*	3*	n	2*	2	2	1	3*	3*	2	3*	3*
	Middle	2	3	n	1	1	1	0	2	1	1	1	2
	Apical	2	0	n	0	0	0	0	0	1	1	0	0

(3) Intense infiltrate; (2) moderate infiltrate; (1) mild infiltrate; (0) absent; (n) pulp necrosis; (*) acute neutrophils-rich infiltrate.

When the presence or absence of mineralized barrier confirmed by histological analysis was compared to the radiographic and tomographic visualization of such mineralizaed structure, there was a coincidence percentage of 79.16% and 87.5%, respectively, as demonstrated in the table 4.

Table 4. Comparative study of the coincidence of the imaginological and histological diagnosis methods for the identification of mineralizad barrier in the experimental and control groups.

Experimental peiod	Diagnosis method	Specimens											
		1	2	3	4	5	6	7	8	9	10	11	12
21 days	RA	+	+	+	+	+	+	+	+	+	-	-	-
	TA	+	+	+	+	+	+	+	+	+	+	-	-
	HA	+	+	+	+	+	+	+	-	-	-	-	-
30 days	RA	+	+	+	+	+	+	-	+	+	+	-	+
	TA	+	+	+	+	+	+	+	+	-	-	-	-
	HA	+	+	+	+	+	+	+	+	+	-	-	-

RA – radiographic analysis; TA – tomographic analysis; HA – histological analysis.

4. Discussion

In humans, the biological effect of pulp protection materials is evaluated fundamentally by the response of the pulp tissue and formation mineralized tissue occluding the exposition zone.

In this study, calcium hydroxide was used as positive control due to its biological properties (biocompatibility, antimicrobian activity, and ability to induce the deposition of newly formed mineralized barrier)[7,8,16,23], and capability of sustaining the vitality of the dental pulp[1,12,13,16,17,28]. Notwithstanding, this product initially causes chemical lesion induced by the release of hydroxyls, leading to superficial necrosis of the pulp tissue. If the pulp is not protected by a suitable marginal sealing, the formation of mineralized barrier may be unsuccessful[3,14,28]. The use of calcium hydroxide induced the formation

of mineralized barrier in all the samples of this study, irrespective the experimental period. Similar findings were reported in previous investigations[1,13], attesting the efficacy of this product as pulp protective material.

It has been demonstrated that the chemical composition of the otoliths, represented by the high content of $CaCO_3$ fixed in an organic matrix constituted of high molecular weight collagenous proteins (otolin-1 and otoliths matrix protein -1), could provide a possible biological activity associated to the mineralization of bone and dentin tissues[18,29]. Therefore, in the current study, the role played by otoliths extracted from *Cynoscion acoupa* in the process of mineralized barrier in dental pulps experimentally exposed of dogs was assessed.

According to our results, otoliths preparation proved to be successful in stimulating the formation of hard tissue barrier, and there was no significant difference regarding its biological activity. Nevertheless, the mechanism responsible for this success is still unknown. It has been suggested that the otoliths mineralization process is closely related to the participation of collagen molecules, similarly to that mechanism of bone and dentin calcification[26]. Furthermore, it has been demonstrated that the protein otolin-1 found in otoliths presents high homology with type VIII and type X collagen, molecules involved in the mineralization of non calcified protein matrices[9]. Also, otoliths are rich in $CaCO_3$ and other inorganic constituents associated to the mineralization dynamics[4]. Hence, it is possible that otolin-1 works as nucleation site for calcium deposition and then facilitate the calcification process using $CaCO_3$ just like collagen molecules propitiate the mineralization of bone and dentin using $CaPO_4$[18]. The histological observation of osteoid formation surrounding basophilic nuclei resembling dystrophic calcification seems to support this theory. In addition, the otoliths alkaline pH (8.77) might also interfere in the hard tissue barrier formation, since it is in between the ideal range for alkaline phosphates activation (8.6 – 10.3)[7]. Notwithstanding, further studies are required to clarify the role played by otoliths in the process of pulp mineralized barrier.

Mild to moderate inflammatory reaction was observed in HC and OTL, in opposition to some previous reports, asserting that calcium hydroxide does not induce pulp inflammatory response[10,12,13,23]. However, it is well-established in the literature that this pulp capping material releases OH ions in the pulp tissue, a highly toxic free radical. Besides, the contact of calcium hydroxide with the pulp connective tissue is supposed to produce areas of superficial necrosis[7,8,16]. As long as necrotic tissues and free radicals work as irritant agents, the presence of an inflammatory response is quite expected. Besides, the low magnitude of this response is suggestive that no major

damage capable to interfere negatively in the regenerative potential of the pulp tissue has occurred.

Despite the suitable results observed in this study, it must be remembered that the otoliths preparation was used in healthy dental pulp. Therefore, further investigations are required in order to find if the same positive responses will be observed in previously infected and inflamed dental pulp.

The identification of mineralized barrier could be accomplished either by radiographic examination or by volumetric cone beam computed tomography, but the latter showed the highest coincidence result index with the histological findings. The effectiveness of cone beam CT has been suggested in recent studies[30]. Furthermore, these data seem to suggest that, given the impossibility of histology, computed tomography with cone beam technology appears to represent a more secure and reliable method for studying the formation of mineralized barrier in dental pulp than the radiological assessment.

5 - Conclusions:

In conclusion, we found that the otoliths preparation is biocompatible and capable to induce the formation of mineralized barrier in pulp similarly to calcium hydroxide. Besides, this work represents an innovative contribution in dental research and it also represents the first report regarding the applicability of otoliths as a quite promising biomaterial to be employed in dentistry. However, further research should be conducted in search of new otoliths formulations for dental direct capping, containing antibiotics, anti-inflammatory and corticosteroids in order to achieve a better performance of this product.

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ANEXOS

Anexo I: Parecer do Comitê de Ética da Universidade Tiradentes

Anexo II: Parecer Preliminar do Comitê de Ética da Faculdade Pio Décimo

Anexo III: Parecer Final do Comitê de Ética da Faculdade Pio Décimo

Anexo IV: Solicitação ao Secretário Municipal de Saúde para cessão dos 2 cães para o experimento

Anexo V: Solicitação ao Diretor da Faculdade Pio Décimo de parceria para o financiamento da pesquisa

Anexo VI: Normas da revista

Anexo I: Parecer do Comitê de Ética da Universidade Tiradentes

Parecer Consubstanciado de Projeto de Pesquisa

Título do Projeto: UTILIZAÇÃO DE OTÓLITOS EM POLPAS DENTAIS DE CÃES PÓS-PULPOTOMIA: UM ESTUDO EXPERIMENTAL COMPARATIVO

Pesquisador Responsável Daisy Pereira Valido

Data da Versão 06/05/2009	Cadastro 030509	Data do Parecer 03/06/2009

Grupo e Área Temática III - Projeto fora das áreas temáticas especiais

Objetivos do Projeto

OBJETIVO GERAL:

Avaliar comparativamente com o hidróxido de cálcio e o MTA a ação de biominerais (otólitos de pescada amarela) no processo de neoformação dentinária após exposição pulpar artificialmente induzida em cães.

OBJETIVO ESPECÍFICO:

- **Analisar radiograficamente a barreira dentinária formada após a aplicação do hidróxido de cálcio, do agregado mineral de trióxido e de otólitos em polpas dentárias expostas de cães.**
- **Analisar histomorfologicamente a barreira dentinária formada após a aplicação do hidróxido de cálcio, do agregado mineral de trióxido e de otólitos em polpas dentárias expostas de cães.**
- **Analisar morfometricamente a barreira dentinária formada após a aplicação do hidróxido de cálcio, do agregado mineral de trióxido e de otólitos em polpas dentárias expostas de cães.**
- **Comparar a eficácia da atividade de neoformação dentinária dos materiais testados em polpas expostas de cães.**

Sumário do Projeto

O hidróxido de cálcio (HC) ainda é o material mais aceito para proteção pulpar direta, sabe-se que quando é colocado em contato com o tecido da polpa preserva a sua vitalidade, sem provocar resposta inflamatória, estimulando a formação de barreira de tecido dentinário mineralizado (BRISO et al, 2006; TORRES et al, 2000). O agregado mineral de trióxido (MTA) é um cimento dental recomendado para o selamento marginal de retrocavidades entre os dentes e os tecidos peridentais. Otólitos são concreções calcárias do ouvido interno dos peixes ósseos, ricos em carbonato de cálcio e em uma proteína colagênica de alto peso molecular denominada otolina (CAMPANA; NELSON, 1984). Adicionalmente, existem nestas estruturas, além do carbonato cálcio, elementos metálicos e não metálicos (BORELLI et al, 2003). Considerando as características físico-químicas dos otólitos e sua potencialidade de ação como biomineral, constitui proposição deste projeto, avaliar a possível atividade estimulatória destes biominerais sobre a formação de barreira dentinária e sua aplicação clínica como material de capeamento pulpar direto. Utilizar-se-á 02 cães, os quais anestesiados e após o isolamento absoluto do campo operatório, realizar-se-á exposição padronizada da polpa, proteção com os materiais capeadores testados e selamento das cavidades com Ionômero de vidro. Após 15 e 30 dias, os dentes serão extraídos, as peças processadas para a análise microscópica dos dentes e observação histomorfológica da barreira dentinária formada. Diante do narrado, justifica-se por propor-se a avaliar comparativamente histomorfológica, radiográfica e morfometricamente com o hidróxido de cálcio e o MTA a ação de biominerais (otólitos de pescada amarela) no processo de neoformação dentinária após exposição pulpar propositadamente induzida, espera-se concluir que esta técnica é de fácil execução, baixo custo, com sucesso previsível e deve ser indicada nos serviços públicos de saúde pelo seu caráter socioeconômico, contribuindo para diminuição do alto índice de perdas dentárias precoces.

Itens Metodológicos e Éticos	Situação
Título	**Adequado**
Autores	**Adequados**
Local de Origem na Instituição	**Adequado**

Bárbara Lima Simioni Leite
Coord. Comitê de Ética em Pesquisa
Universidade Tiradentes

Projeto elaborado por patrocinador	Não
Aprovação no país de origem	Não necessita
Local de Realização	Própria instituição
Outras instituições envolvidas	Não
Condições para realização	Adequadas

Comentários sobre os itens de Identificação

RESUMO - o resumo apresenta-se bem caracterizado. Contudo, chamamos atenção para a desnecessidade da utilização de citações no corpo dessa etapa do trabalho.

Introdução	Adequada

Comentários sobre a Introdução

Objetivos	Adequados

Comentários sobre os Objetivos

Pacientes e Métodos	
Delineamento	Adequado
Tamanho de amostra	Total Local
Cálculo do tamanho da amostra	Adequado
Participantes pertencentes a grupos especiais	Não
Seleção eqüitativa dos indivíduos participantes	Adequada
Critérios de inclusão e exclusão	Adequados
Relação risco- benefício	Não apresentada
Uso de placebo	Não utiliza
Período de suspensão de uso de drogas (wash out)	Não utiliza
Monitoramento da segurança e dados	Ausente
Avaliação dos dados	Adequada - quantitativa
Privacidade e confidencialidade	Não se aplica
Termo de Consentimento	Não se aplica
Adequação às Normas e Diretrizes	Sim

Comentários sobre os itens de Pacientes e Métodos

Cronograma	Comentário
Data de início prevista	
Data de término prevista	
Orçamento	Adequado
Fonte de financiamento externa	Não

Comentários sobre o Cronograma e o Orçamento

Referências Bibliográficas	Adequadas

Comentários sobre as Referências Bibliográficas

Recomendação

Aprovar

Comentários Gerais sobre o Projeto

O presente trabalho apresenta grande relevância clínica e seus resultados poderão contribuir em muito para o tratamento das afecções pulpares de grande ocorrência na população.

Bárbara Lima Simioni Leite
Coord. Comitê de Ética em Pesquisa
Universidade Tiradentes

Anexo II: Parecer Preliminar do Comitê de Ética da Faculdade Pio Décimo

FACULDADE PIO DÉCIMO

Associação de Ensino e Cultura "Pio Décimo" www.piodecimo.com.br
Reconhecida pelo Decreto N.° 83.064 de 22.01.79

MEMORANDO n° 20/09 Aracaju, SE, 01 de Julho de 2009

À Senhora Daisy Pereira Valido

De: Comissão de Ética e Bem-estar Animal Pio X
Assunto: **Projeto de pesquisa UNIT**

Prezada Senhora

De acordo com avaliação prévia da Comissão de Ética e Bem-estar Animal da Faculdade Pio Décimo, venho através deste documento documentar o parecer determinado pela comissão em relação ao projeto de pesquisa intitulado "Utilização de otólitos em polpas dentais de cães pós-pulpotomia: um estudo experimental comparativo" de acordo coma solicitação da Sra. Daisy Pereira Valido.

Após leitura e avaliação do projeto, a comissão determinou a revalidação de alguns itens referentes a metodologia a ser aplicada, tais como:

1. Protocolo anestésico: é extremamente contra-indicado a utilização de tiletamina/zolazepan para procedimentos orais invasivos, principalmente em dentes incisivos devido ao "efeito serpente" dos movimentos da língua bem como pouco ou nenhuma analgesia;
2. Quarentena: como os animais virão da zoonose, terão que ficar em período de quarentena onde além do hemograma, uréia e creatinina deverão ser feitos também função hepática, urinálise, plaquetas e exame de fezes;
3. Pós-operatório: como se trata de um procedimento dentário invasivo, os procedimentos pós-operatórios para controle da dor e infecções é de extrema importância devendo, portanto, estar descriminado no protocolo;
4. Adoção: após o experimento, os animais ficarão sem os dentes incisivos, dificultando ainda mais a adoção desses animais, assim, indica-se um destino mais adequado para os animais.

Assim, indefere-se o projeto sob as condições atuais, onde o mesmo deverá ser novamente submetido para nova avaliação após modificação dos itens supracitados.

Atenciosamente,

Prof. MSc. Marcio de Castro Menezes
Médico Veterinário
Membro da Comissão - Faculdade Pio X

Prof. MSC. Márcio C. Menezes
CRMV-SE: 0542

Anexo III: Parecer Final do Comitê de Ética da Faculdade Pio Décimo

FACULDADE
PIO DÉCIMO

Associação de Ensino e Cultura "Pio Décimo"
Reconhecida pelo Decreto N.° 83.064 de 22.01.79

www.piodecimo.com.br

MEMORANDO n° 02/09 Aracaju, SE, 18 de Agosto de 2009

À Senhora
Daisy Pereira Valido

De: Comissão de Ética e Bem-estar Animal Pio X

Assunto: **Projeto de pesquisa UNIT**

Prezada Senhora

A Comissão de Ética e Bem-estar Animal da Faculdade Pio Décimo vem através deste documento **DEFERIR** o processo referente ao projeto encaminhado para avaliação intitulado "*Utilização de otólitos em polpas dentais de cães pós-pulpotomia: um estudo comparativo*". A partir desse momento, a Sra. Daisy Pereira Valido ficará na responsabilidade de entregar à Comissão a ficha de adoção dos animais utilizados no experimento.

Atenciosamente,

Prof. MSc. Marcio de Castro Menezes
Médico Veterinário
Membro da Comissão de Ética- Faculdade Pio X

Prof. MSC. Márcio C. Menezes
CRMV-SE: 0542

Anexo IV: Solicitação ao Secretário Municipal de Saúde para cessão dos 2 cães para o experimento

Exmo Sr. Dr. MARCOS RAMOS CARVALHO,
Secretário Municipal de Saúde

Eu, Daisy Pereira Valido, brasileira, casada, maior capaz, servidora pública federal, cirurgiã-dentista, portadora da cédula de identidade 575.255 SSP-SE, CPF 42382394587, título de eleitor 156.20.21.51, estudante do curso de mestrado em Saúde e Ambiente da Universidade Tiradentes, venho, por meio deste, solicitar a cessão de 02 (dois) cães do Centro de Controle de Zoonoses de Aracaju como modelo experimental, para a realização do experimento da minha tese de mestrado, que tem como tema de dissertação a "UTILIZAÇÃO DE OTÓLITOS EM POLPAS DENTAIS DE CÃES PÓS-PULPOTOMIA: UM ESTUDO EXPERIMENTAL COMPARATIVO", cujo projeto de pesquisa encontra-se aprovado pelo comitê de ética da Universidade Tiradentes, conforme projeto e parecer em anexos. Em tempo, informo que a realização do experimento dar-se-á em parceria com a Faculdade Pio Décimo, sob a supervisão de médico veterinário.

Nestes termos, aguardo deferimento,

Aracaju, 16 de julho de 2009

Daisy Pereira Valido

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17-07-09

3106-9711
Eleni

Anexo V: Solicitação ao Diretor da Faculdade Pio Décimo de parceria para o financiamento da pesquisa

Exmo Sr. Prof. JOSÉ SEBASTIÃODOS SANTOS,
Diretor Geral da Faculdade Pio Décimo

Eu, Daisy Pereira Valido, brasileira, casada, maior capaz, servidora pública federal, cirurgiã-dentista, portadora da cédula de identidade 575.255 SSP-SE, CPF 42382394587, título de eleitor 156.20.21.51, estudante do curso de mestrado em Saúde e Ambiente da Universidade Tiradentes, venho, por meio deste, solicitar a autorização e a parceria da Faculdade Pio Décimo para a utilização do seu Hospital Veterinário na realização do experimento da minha tese de mestrado, no qual serão utilizados 02 (dois) cães provenientes do Centro de Controle de Zoonoses de Aracaju como modelo experimental e que tem como tema de dissertação a "UTILIZAÇÃO DE OTÓLITOS EM POLPAS DENTAIS DE CÃES PÓS-PULPOTOMIA: UM ESTUDO EXPERIMENTAL COMPARATIVO", cujo projeto de pesquisa encontra-se aprovado pelo comitê de ética da Universidade Tiradentes e em análise pela Comissão de Ética e Bem-estar Animal da Faculdade Pio Décimo que, de acordo com sua avaliação prévia, determinou a revalidação de alguns itens referentes à metodologia a ser aplicada, dentre eles a quarentena dos animais para que os mesmos fiquem em condições ideais de saúde e realizem exames (hemograma, uréia, creatinina, função hepática, urinálise, plaquetas e exame de fezes), de modo a garantir que os resultados da pesquisa não sejam alterados pelas condições de saúde/doença do animal. Após a quarentena, os cães, sob anestesia geral, serão submetidos à profilaxia, limpeza de tártaro e alisamento manual, ao isolamento absoluto do campo operatório com dique de borracha, exposição padronizada da polpa de dentes hígidos, proteção pulpar com os materiais capeadores a serem testados e ao selamento das cavidades com restauração de ionômero de vidro fotopolimerizável. Os animais, após concluída a parte experimental, serão mantidos em cativeiros individualizados por até 30 dias, com alimentação pastosa e água à vontade. Após 15 e 30 dias, os animais 1 e 2 respectivamente, serão anestesiados, os incisivos extraídos e as peças serão processadas, no laboratório de patologia da UNIT, para a análise microscópica dos dentes e observação histomorfológica da barreira

Daisy

dentinária formada. Tendo em vista a quarentena, os 31 dias para a realização do experimento, o período de recuperação pós-operatório dos animais, os exames necessários, o porte anestésico e o uso das instalações do centro cirúrgico, solicito a parceria da Faculdade Pio Décimo nos gastos necessários para a realização do experimento. Em tempo, informo que o experimento dar-se-á sob a supervisão dos professores do curso de Medicina Veterinária da Faculdade Pio Décimo e com o acompanhamento clínico dos animais pela médica veterinária Suelem Mendes da Mota Bravo, ex-aluna do curso de medicina veterinária dessa Faculdade.

Nestes termos, aguardo deferimento,

Aracaju, 6 de agosto de 2009

Daisy Pereira Valido

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ANEXO VI: Normas da revista

JOURNAL OF APPLIED ORAL SCIENCE

ISSN 1678-7757 *printed version*
ISSN 1678-7765 *online version*

INSTRUCTIONS TO AUTHORS

Scope and policy

1 SCOPE

The **Journal of Applied Oral Science** is committed in publishing the scientific and technologic advances achieved by the dental community, according to the quality indicators and peer reviewed material, with the objective of assuring its acceptability at the local, regional, national and international levels. The primary goal of The Journal of Applied Oral Science is to publish the outcomes of original investigations as well as invited case reports and invited reviews in the field of Dentistry and related areas.

2 General Guidelines

2.1 The papers sent for publication must be original and the simultaneous submission to other journal, either national or international, is not allowed. The Journal of Applied Oral Science shall retain the copyright of all papers published, including translations, yet allowing future reproduction as a transcription, provided the source is properly mentioned.

2.2 Only papers written in the English language shall be accepted, and the authors are fully responsible for the texts, citations and references.

2.3 The Journal of Applied Oral Science has the right to submit all manuscripts to the Editorial Board, which is fully authorized to settle the convenience of their acceptance, or return them to the authors with suggestions for modifications in the text and/or for adaptation to the editorial rules of the Journal. In this case, the manuscript will be re-evaluated by the Editor-in-Chief and Editorial Board.

2.4 The concepts stated on the papers published are full responsibility of the authors and do not necessarily reflect the opinion of the Editor-in-Chief and Editorial Board.

2.5 The dates of receipt of the original paper and its acceptance will be indicated in the occasion it is published.

2.6 Each author will receive one copy of the Journal. Additional reprints may be supplied upon request and must be paid by the authors.

2.7 Depending on the financial resources of the Journal of Applied Oral Science or the authors, color illustrations will be published at the discretion of the Editor-in-Chief.

3 Revision Criteria

3.1 Manuscripts will be firstly evaluated regarding presentation according to the instructions for authors. Manuscripts not in accordance with instructions will be rejected and returned to authors without being reviewed by referees.

3.2 Manuscripts in accordance with the instructions will be appreciated in their scientific merit and methods by at least two referees from different institutions of that of the authors, besides the Editor-in-Chief. When revision of the original is required, the manuscript will be returned to the corresponding author for modification. A revised version with modifications will be re-submitted by the

authors, and that will be re-evaluated by the Editor-in-Chief and Editorial Board.
3.3 The Editor-in-Chief will decide upon the acceptance of the manuscript, and may return it to the authors for revision and necessary modifications in the text and/or illustrations. In this case, the authors will be required to re-submit a revised version with the modifications or proper explanations. The revised version will be reviewed by the Editor-in-Chief and Editorial Board.
3.4 Upon approval of the scientific merit, manuscripts will be analyzed regarding the use of proper English grammar (technical review) and statistics. If manuscripts are still considered inadequate, they will be returned to authors for revision.
3.5 Authors and referees will be kept anonymous during the review process.
3.6 Contents of the manuscript are the authors' responsibility and do not reflect the opinion of the Editor-in-Chief or Editorial Board.

4 Galley Proof

4.1 Galley proofs will be sent to the corresponding author by electronic mail in pdf format for final approval.
4.2 Approval of galley proofs by the corresponding author should be returned with corrections, if necessary, within 72 hours.
4.3 If not returned within 72 hours, the Editor-in-Chief will consider the present version the final, and will not allow further modifications. Corrections in the galley proofs should be restricted to minor mistakes that do not modify the content of the manuscript. Major corrections will imply that the manuscript should enter the review process again.
4.4 Inclusion of new authors is not allowed at this phase of the publication process.

Form and preparation of manuscripts

1 Presentation of the Manuscript

1.1 Structure of the manuscript

- Cover page (must be submitted as a supplementary file through the online submission system) which should contain only:
- Title of the manuscript in English.
- Names of the authors in direct order with their respective degrees and affiliations in English. Correspondence between International and Brazilian degrees may be obtained at our web page: www.fob.usp.br/jaos.
- Full address of the corresponding author, to whom all correspondence should be addressed, including fax and phone number as well as e-mail address.

1.2 Text

- Title of the manuscript and subtitle, if necessary, in English.
- Abstract: should comprise at most 300 words, highlighting a little introduction, objective, material and methods, results and conclusions.
- Key words: (words or expressions that identify the contents of the manuscript). The authors are referred to the list of subjects of the "lndex Medicus" and DeCS (Health Sciences Descriptors available at http://decs.bvs.br/). Authors must use "periods" to separate the key words, which must have the first letter of the first word in capital letters. Ex: Dental implants. Fixed prosthesis. Photoelasticity. Passive fit.
- Introduction: summary of the rationale and proposal of the study including only proper references. It should clearly state the hypothesis of the study.
- Material and Methods: the material and the methods are presented with enough detail to allow confirmation of the findings. Include city, state and country of all manufacturers right after the first appearance of the products, reagents or equipments. Published methods should be referred to and briefly discussed, except if modifications were made. Indicate the statistical methods employed, if applicable. Please refer to item 3 for ethical principals and registration of clinical trials.
- Results: presents the outcomes in a logical sequence in the text, tables and illustrations. Data contained in tables and illustrations should not be repeated in the text, and only important findings should be highlighted.
- Discussion: this should emphasize the new and important aspects of the study and the resulting conclusions. Any data or information mentioned in the

introduction or results should not be repeated. Findings of other important studies should be reported. The authors should point out the implications of their findings as well as their limitations.
- Conclusion(s) (if any)
- Acknowledgments (when appropriate). Acknowledge those who have contributed to the work. Specify sponsors, grants, scholarships and fellowships with respective names and identification numbers.
- References (please refer to item 2.3)

2 TECHNICAL NORMALIZATION

The manuscript should be typed as follows: 1.5 spacing in 11 pt Arial font, with 3-cm margins at each side, on an A4 page, adding up to at most 15 pages, including the illustrations (graphs, photographs, tables, etc). The authors should keep a copy of the manuscript for possible requests.

2.1 Illustrations and Tables

2.1.1 The illustrations (photographs, graphs, drawings, charts, etc.), regarded as figures, should be limited to the least amount possible and should be uploaded in separate files, consecutively numbered with Arabic numbers according to the order they appear in the text.

2.1.2 Photographs should be sent in original colors and digitized in .jpg, tif or gif formats with **10cm width and at least 300dpi**. These illustrations should be provided in supplementary files and **not inserted in the Word document**.

2.1.3 The corresponding legends for figures should be clear, concise and typed at the end of the manuscript as a separate list preceded by the corresponding number.

2.1.4 The tables should be logically arranged, consecutively numbered with Arabic numbers. The legend shall be placed on the top of the tables. Tables should be open in the right and left laterals.

2.1.5 Footnotes should be indicated by asterisks and restricted to the least amount possible.

2.2 Citation of the Authors

Citation of the authors in the text may be performed in two manners:

1) Just numeric: … and interfere with the bacterial system and tissue system [3,4,7-10]. References must be cited in a numeric ascending order within the paragraph.

2) or alphanumeric

- one author - Silva[23] (1986)
- two authors - Silva and Carvalho[25] (1987)
- three authors - Ferreira, Silva and Martins[27] (1987)
- more than three authors- Silva, et al.[28] (1988)

Punctuation characters such as "periods" and "commas" must be placed after the numeric citation of the authors. Ex: Ferreira[38].

2.3 References

The references must follow the Uniform requirements for manuscripts submitted to Biomedical Journals – Vancouver available at: http://www.nlm.nih.gov/bsd/uniform_requirements.html.

2.3.1 All references must be cited in the text. They should be **alphabetically ordered** by the last name of the author and numbered in increasing order accordingly. The order of citation in the text should follow these numbers. Abbreviations of the titles of the international journals cited should follow the Index Medicus/MEDLINE.

2.3.2 Personal communications and unpublished data with no publication date must not be included in the reference list.

2.3.3 Abstracts, monographs, dissertations and theses will not be accepted as references.

2.3.4 The names of all authors should be cited up to 6 authors; in case there are more authors, the 6 first authors should be cited, followed by the expression ", et al.", which must be followed by "period" and should not be written in italics. Ex: Uhl, et al.

2.3.5 At most 30 references may be cited, except for invited reviews by the Editor-in-Chief.

Examples of references:

Book
Melberg JR, Ripa LW, Leske GS. Fluoride in preventive dentistry: theory and clinical applications. Chicago: Quintessence; 1983.
Book chapter
Verbeeck RMH. Minerals in human enamel and dentin. ln: Driessens FCM, Woltgens JHM, editors. Tooth development and caries. Boca Raton : CRC Press; 1986. p.95-152.
Papers published in journals
Wenzel A, Fejerskov O. Validity of diagnosis of questionable caries lesions in occlusal surfaces of extracted third molars. Caries Res. 1992;26:188-93.
Papers with more than 6 authors
The first 6 authors are cited, followed by the expression ", et al."
Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood - leukemia in Europe after Chernobyl : 5 years follow-up. Br J Cancer. 1996;73:1006-12.
Papers without authors' names
Seeing nature through the lens of gender. Science. 1993;260:428-9.
Volume with supplement and/or Special Issue
Davisdson CL. Advances in glass-ionomer cements. J Appl Oral Sci. 2006;14(sp. Issue):3-9.
Entire issue
Dental Update. Guildford 1991;18(1).
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3 ETHICAL PRINCIPLES AND REGISTRATION OF CLINICAL TRIALS

3.1 Experimental procedures in humans and animals

The Journal of Applied Oral Science reassures the principles incorporated in the Helsinky Declaration and insists that all research involving human beings, in the event of publication in this journal, be conducted in conformity with such principles and others specified in the respective ethics committees of authors' institution. In the case of experiments with animals, such ethical principles must also be followed. When surgical procedures in animals were used, the authors should present, in the Material and Methods section, evidence that the dose of a proper substance was adequate to produce anesthesia during the entire surgical procedure. All experiments conducted in human or animals must accompany a description, in the Material and Methods section, that the study was approved by the respective Ethics Committee of authors' affiliation and provide the number of the protocol approval. The Editor-in-Chief and the Editorial Board reserve the right to refuse manuscripts that show no clear evidence that the methods used were not appropriate for experiments in humans or animals.

3.2 Clinical Trial Registration - International Standard Randomized Controlled Trial Number (ISRCTN)

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In order to register a clinical trial, please access one the following addresses:
Register in the **Clinicaltrials.gov**
URL: http://prsinfo.clinicaltrials.gov/
Register in the **International Standard Randomized Controlled Trial Number (ISRCTN)**

URL: http://www.controlled-trials.com/

4 ANY QUERIES SHALL BE SOLVED BY THE Editor-in-Chief AND EDITORIAL BOARD

Sending of manuscripts

1 MANUSCRIPT SUBMISSION

1.1 Articles must be submitted through the following address http://www.scielo.br/jaos

1.2 The corresponding author should retain the original file in Word format as well as illustrations (when applicable).

1.3 The original file containing the main manuscript must be submitted without the authors' identification and affiliations. The cover page must be submitted as a supplementary file containing the names of the authors, affiliations and correspondence address.

1.4 Figures must be submitted as supplementary files according to the specifications of item 2.1 regarding the form and preparation of manuscripts.

1.5 Tables must be prepared in Word format and inserted after the references at the end of the original Word file.

1.6 The submission Form, signed by ALL the authors, must be submitted as a supplementary file containing the following text:

By signing the Submission Form, the authors state:

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