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Effects of Chitosan as a Novel Obturation Material for Pulpectomized Teeth on Periapical Inflammation, Periodontal Ligament Widening, and Hard Tissue Resorption: A Preliminary Exploratory Study on Dogs

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A B S T R A C T

Objectives: This preliminary animal study was conducted to assess the effects of chitosan as a novel obturation material for pulpectomized teeth on periapical inflammation, periodontal ligament (PDL) widening, and hard tissue resorption.

Materials and Methods: Forty premolar root canals in two mature dogs were obturated with zinc oxide eugenol (ZOE) and an experimental 3% chitosan paste (n=20 in each group). The teeth were then restored with amalgam. After 28 days, the dogs were sacrificed, and histopathological assessment was performed. The amount of resorbed obturation material, degree of inflammatory response, degree of PDL widening, and the number of bone/cementum/dentin resorption defects were recorded under ×40 and ×200 magnifications. Data were analyzed using the Mann-Whitney U test, one-sample Wilcoxon signed-rank test, and Fisher's exact test (α=0.05).

Results: Bone, cementum, and dentin resorption were seen in 6, 10, and 1 chitosan-obturated canals and 14, 15, and 0 ZOE-obturated canals, respectively. Only the bone resorption defects were significantly fewer in the chitosan group (P=0.026). Mild, moderate, and severe inflammation were observed in 17, 3, and 0 chitosan-obturated canals, and 7, 9, and 4 ZOE-filled canals, respectively (P=0.004). Mild, moderate, and severe PDL widening were seen around 15, 5, and 0 chitosan-filled canals and 7, 12, and 1 ZOE-filled canals, respectively (P=0.025).

Conclusion: The 3% chitosan was superior to ZOE in terms of causing less inflammation and PDL widening. It also decreased bone resorption and acted similar to ZOE in terms of dentin and cementum resorption.

Keywords: Chitosan; Zinc Oxide-Eugenol Cement; Pulpectomy; Inflammation; Periodontal Ligament

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INTRODUCTION

The goal of root canal treatment in permanent or primary dentition (i.e., pulpectomy) is to mechanically and chemically remove the contaminated tissues, bacteria, and bacterial products from the root canal system [1,2]. Although mechanical debridement is an important part of root canal therapy, it cannot

decrease the bacterial load of the canal by more than one-half, because anatomical limitations (e.g., isthmi and apical accessory canals) do not allow the instruments to reach all areas of the canal. Such limitations and anatomical variations are more noticeable in primary teeth with thinner roots and root walls, which also undergo physiological resorption [1-4]. Due to such anatomical limitations and the subsequently low success rate of mechanical instrumentation alone, antimicrobial agents are used to aid in further removal of bacteria, debris, and infected and necrotic tissues [1,2,5]. Nevertheless, chemo-mechanical preparation and debridement of the canal still fail to completely remove the bacteria and their products from the root canal system [1,6]. Thus, more precautionary measures need to be taken in order to ensure that the residual bacteria and their products do not leach into the periapical tissues and cause inflammation and infection; such measures may include sealing of the canal and apex with antimicrobial obturation materials [1,4].

Developmental, anatomical, and physiological differences between the primary and permanent teeth lead to different standards for obturation materials [7]. An ideal root canal filling material should easily fit and fill the canal and adhere to the root canal walls, must be antiseptic, should not undergo shrinkage, must be biocompatible and safe for the periapical tissues and permanent tooth buds, must be highly resorbable if pushed beyond the apex, should be radiopaque, should not cause dental discoloration, and must be easily washable and retrievable, if required [8]. An ideal obturation material needs to be biocompatible because it may leak through the apex into the periapical tissues and distribute locally and systemically [1]. In primary dentition, an ideal obturation material should undergo resorption at a similar rate to the speed of physiological root resorption [1,9]. The most commonly used materials for obturation of primary root canals include zinc oxide eugenol (ZOE), iodoform compounds, and calcium hydroxide [1,4]. ZOE is the most commonly used obturation material recommended by the American Academy of Pediatric Dentistry for use as an obturation material for primary roots [1,10]. It has an average success rate of 83% (range: 65% to 100%) with no significant difference with the results of iodine- or calcium hydroxide-based pastes [1,9].

Although the commonly used obturation materials for primary roots have numerous advantages, they also have limitations; for example, ZOE and KRI pastes can be cytotoxic for 1 and 7 days after setting, respectively [1,11]. Furthermore, due to the thinness of the root canal walls of primary teeth, ZOE is likely to be overfilled and exit through the apex, which can considerably reduce the success rate from 58% to 83% [1,12]. ZOE also has other disadvantages such as slow resorption rate, irritation of periapical tissues, bone and cementum necrosis, and alteration of the eruption path of permanent teeth [10]. Other common obturation materials have their own drawbacks, such as allergy and encephalopathy followed by coma in use of iodoform-containing compounds [1,13,14].

Thus, a new obturation material that can provide the advantages of previous commonly used materials without their disadvantages would be of significant clinical use. Chitosan is produced by deacetylation of chitin, and was recently suggested for different stages of root canal therapy due to its adequate biocompatibility as well as antimicrobial and anti-inflammatory activities [1]. It is a cationic polysaccharide which is non-toxic and highly biocompatible [1,15]. Its positive charge allows its attachment to the negatively charged surface of bacteria or fungi, increasing their permeability and facilitating their destruction [1,2,16]. It can eliminate a wide range of fungi and bacteria, and is effective against viruses and even tumoral cells. It also has anti-inflammatory properties, boosts the immune system, induces tissue regeneration, and affects Sn2+ enhancing its anti-erosive and anti-abrasive effects [1,2,17-21]. It is also easily available since its base substance, chitin, is a major component of the exoskeletons of the crustaceans and the second most available biopolymer in nature, after cellulose [1,2,22].

Due to its antibacterial and anti-demineralization activities, chitosan has been applied in various products such as dentifrices

and chewing gums, and has been effective in reducing enamel decalcification and caries as a result of its antimicrobial activity [1,2,15,23,24]. It has also been tested as irrigant in root canal therapy [2,25-27] and as an obturation material [1]. In addition to its antimicrobial activity, chitosan was recently shown to be capable of optimally filling and sealing the root canal space [1]. The aforementioned in vitro evidence suggests chitosan as a possibly good obturation material for pulpectomy. However, studies in this regard have been very few, especially in primary dentition. Moreover, no in vivo study is available on chitosan as a root canal filling material. Therefore, this preliminary study aimed to experimentally produce a chitosan-containing obturation material for pulpectomy and assess its biocompatibility in dogs' teeth.

MATERIALS AND METHODS

Phase 1: Preparation of 3% chitosan paste

Initially, 0.15 g of 1% acetic acid (BDH, England) was mixed with 50g of chitosan powder (Sigma, USA) with a viscosity of less than 25 P. The compound was kept in a closed container for 24 hours to fully hydrate the chitosan. Barium sulfate was used to make the solution opaque: first, 15g of chitosan suspension was poured into a bottle. Afterwards, 1.5 g barium sulfate (10% of chitosan weight) was gradually added to the suspension until a uniform paste was obtained. Then, the remainder of the chitosan suspension was added to the mixture and blended (Ultra Homogenizer, IKA, Germany) for one hour to obtain a perfectly homogenous paste. The resulting material had a fairly uniform and relatively thick consistency, with an estimated pH of 3.8 (pH Meter, Presens, Germany).

Phase 2: Pilot study

Two extracted human primary canine teeth were selected for the pilot study. Access cavity was prepared using diamond burs. The canals were instrumented with K-files (Mani-Korea) #20, 25, 30, and 35. After using each file, the canals were irrigated with saline. Then, the prepared canals were filled with the obtained paste by the injection method. Finally, periapical radiographs were obtained from the teeth to confirm proper obturation of the canals.

Phase 3: Animal study

Animals were selected in accordance with the ethical guidelines of the Animal Care and Use Committee. The protocol of the study was approved by the Ethics Committee of the Research Center on Laboratory Animals (IR.AJUMS.REC.1395.133). The dogs were kept in a standard room in the animal house of the Dental Research Center of Isfahan University of Medical Sciences. The dogs were provided with sufficient water and fed dry food.

The dogs were obtained from the dog breeding centers, examined by a veterinarian, and vaccinated after admission. Antiparasitic treatment was also performed for them [28]. The dogs were kept in quarantine for 14 days, and were re-examined twice by the veterinarian during this period. After the quarantine period, they were re-examined once more and after ensuring their health, they entered the main animal house.

A mature dog has 42 teeth which erupt completely within 7 months. A dog has 3 premolars in each quadrant [29]. The first premolar teeth of dogs have one canal, and their second and third premolars have two canals each. The first, second and third mandibular and maxillary premolars of two 18-month-old dogs (Iranian mixed hybrid breed) were selected for pulpectomy (a total of 40 canals in 12 teeth). This sample size was determined based on discussion to have a reasonable sample size of the canals and to sacrifice the minimum number of animals. These 40 canals were divided into 4 groups of 10 each:

Group A: 6 upper and lower right premolars (10 root canals) of the first dog that were obturated with 3% chitosan paste (Sigma, USA).

Group B: 6 upper and lower left premolars (10 root canals) of the first dog that were obturated with ZOE paste (KemDent, UK).

Group C: 6 upper and lower right premolars (10 root canals) of the second dog that were obturated with ZOE paste.

Group D: 6 upper and lower left premolars (10 root canals) of the second dog that were obturated with 3% chitosan paste.

The dogs were anesthetized with 0.2cc/kg of 1% acepromazine (Petmeds, USA) and 10mg/kg ketamine (Rotexmedica, Germany) [30]. After

irrigation with 2% chlorhexidine (Najo, Tehran, Iran), the access cavity was prepared in the occlusal surface, and the working length was estimated 2mm shorter than the apex on periapical radiographs using a #20 K-file (Mani, Japan). The teeth were filed up to #40 file according to the standard technique. After using each file, the root canals were rinsed with a minimum of 3.6 mL of saline. If there was still debris in the canal, the filing would continue after the irrigation. After washing, the canals were dried with sterile paper points (Mani, Japan). The root canals were obturated by the injection technique.

All teeth were restored with amalgam (GK116, China). Next, for evaluation of the quality and extent of obturation, periapical radiographs were obtained from all canals (AGFA, Japan). Overfilled canals had to be excluded from the study (there was no overfilled canal to be excluded). After 28 days [31], periapical radiographs were obtained again to evaluate root canal obturations.

The dogs were then sacrificed by the vital perfusion method, which enables rapid fixation of tissues before any autolysis and necrosis after the death of animal in order to have a normal view of the tissues and perform more precise cellular studies. The animals were anesthetized and the cervical vein and the carotid artery were identified on both sides of the neck using the carotid pulse. The vessels were clamped, and a catheter was inserted into the carotid artery from the clamped section. The blood within arteries was removed using normal saline. Afterwards, 10% formalin solution was injected into the carotid catheter. At the end of this phase, while the heart rate was low, the carotid artery was clamped beneath the clogged location and jugular vein. The heartbeat stopped by removal of blood from the body and the induced hypovolemic state.

The jaws were surgically removed, washed with tap water, and coded according to the number of dogs and teeth (in terms of filling material) and placed in formalin.

Phase 4: Histopathological assessment

The specimens were stored in formalin for 24 hours (Chembid, China). Afterwards, they were stored in 10% formic acid (Merck, Germany) for 30 days to decalcify and soften the jaws for easier cutting. The acid was refreshed once a week to remove calcium deposits. For processing, the tissues were diced into small pieces and immersed in 50% ethanol (Merck, Germany). The samples were subjected to ascending concentrations of alcohol up to 100% concentration. The last container had xylol alcohol (Merck).

The specimens were cut into 5-µm long sections by a microtome (YD-1512, USA) and stained with hematoxylin (Kerr, Germany), eosin (Kerr, Germany) and Mallory's trichrome stain (Kerr, Germany). The periapical areas were inspected by one observer under a light microscope (CX21; Olympus, Tokyo, Japan). A magnification of ×40 was used to assess the thickness of the periodontal ligament (PDL) and to analyze the hard tissues. Inflammation was assessed at ×200 magnification.

Primary outcome: Histopathological variables

The degree of inflammation was categorized as follows:

Mild: presence of three parameters of edema, vasodilatation, and maximum inflammatory cells in 4 microscopic fields.

Moderate: Presence of at least 2 of the above-mentioned parameters in at least 5 and at most 9 microscopic fields.

Severe: Presence of at least 2 of the above-mentioned parameters in at least 10 microscopic fields [32].

The normal PDL width is 0.15-0.2mm. In the present study, increased PDL thickness was categorized as follows:

Mild: Increased PDL width by 0.2-0.3mm.

Moderate: Increased width more than 0.3-0.4mm.

Severe: Increased width by 0.4-0.5mm [32].

Specimens were also assessed in terms of the predominant types of white blood cells [3]. Another assessed parameter was the presence or absence of hard tissue resorption (bone, dentin, cementum).

Statistical analysis

The Mann-Whitney U test was used to compare the inflammation scores between the chitosan and ZOE groups. It was also used to compare the two groups regarding PDL widening. The Fisher's exact test was used to compare the

groups in terms of dichotomous variables namely bone resorption, cementum resorption, and dentin resorption. One-sample Wilcoxon signed-ranks test was used to compare the inflammation scores or PDL widening scores in each material group compared to the moderate score of 2 for inflammation or PDL widening. Significance was set at a 0.05 level.

RESULTS

Periapical radiographs taken 28 days after pulpectomy showed that most of the chitosan was resorbed while most of the ZOE had remained (Fig. 1). The dominant inflammatory cells were macrophages and lymphocytes in the chitosan group, while neutrophils were dominant in the ZOE group.

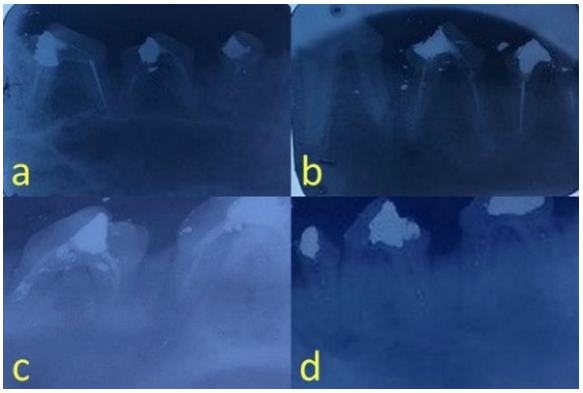

Fig.1: Periapical radiographs showing ZOE (a) and chitosan (b) immediately after the treatment, and the remaining ZOE (c) and the resorbed chitosan (d) 28 days after the treatment

Hard tissue resorption

As shown in Table 1, there was no significant difference in the frequency of cementum resorption (P=0.191) or dentin resorption defects (P=1) between the chitosan and ZOE groups. However, the chitosan group showed significantly lower bone resorption than the ZOE group (P=0.026).

Table 1. Frequency distribution of hard tissue resorption in the two groups (N=20)

Tissue	Chitosan N(%)	ZOE N(%)	P*
Bone	6(30)	14(70)	0.026
Cementum	10(50)	15(75)	0.191
Dentin	1(5)	0	1

ZOE: zinc oxide eugenol
*Fisher's exact test

Inflammation

The Mann-Whitney U test showed that the extent of inflammation was significantly lower in the chitosan group compared to the ZOE group (P=0.001, Table 2). According to the one-sample Wilcoxon signed-rank test, there was a significant skewness towards mild inflammation in the chitosan group (P<0.0001); while in the ZOE group, all grades of inflammation had a similar frequency and their medians did not differ significantly from the moderate inflammation group (P=0.549, Table 2).

Table 2. Frequency distribution of inflammation grades in the two groups

Inflammation	Chitosan N(%)	ZOE N(%)
Mild	17(85)	7(35)
Moderate	3(15)	9(45)
Severe	0	4(20)

ZOE: zinc oxide eugenol

Periodontal ligament widening

The Mann-Whitney U test showed that the PDL widening scores were significantly lower in the chitosan group compared to the ZOE group (P=0.01, Table 3). In the ZOE group, moderate PDL widening followed by mild widening were much more common than severe widening, but comparing with score 2 (moderate widening), the results did not reach statistical significance (P=0.070, one-sample Wilcoxon, Table 3) meaning that the median PDL widening grade did not significantly differ from moderate. However, in the chitosan group, the distribution of PDL widening tended towards mild widening (P<0.0001, one-sample Wilcoxon, Table 3).

Table 3. Frequency distribution of PDL widening grades in the two groups

PDL widening	Chitosan N(%)	ZOE N(%)
Mild	15(75)	7(35)
Moderate	5(25)	12(60)
Severe	0	1(5)

ZOE: zinc oxide eugenol

DISCUSSION

This study was the first to assess the application of chitosan as an endodontic material in vivo in order to evaluate its

biocompatibility and resorption-inducing behavior. Since there is no other study in this regard (even in vitro), only more general aspects of chitosan are now discussed. Histopathological evaluation of the samples in this study showed no evidence of chitosan infiltration into the periapical area and tissue fluids. Radiographic evaluation of the specimens in the present study after 28 days in both groups showed that chitosan degraded faster than ZOE, and it appears that in human primary teeth, it might resorb faster than a primary tooth would. Chitosan is not radiopaque; thus, in this study, it was mixed with barium sulfate to opacify it without affecting its physical and chemical properties. It also had a positive effect on the viscosity of chitosan. The histopathological findings of the 3% chitosan group in the current study indicated the superior biocompatibility of this material. To the best of the authors' knowledge, no previous study is available on endodontic use of chitosan for the purpose of comparison with the present results. In the present study, one root showed dentinal resorption after the application of chitosan. In a study by Sun et al, [17] the pulp cap paste containing chitosan was in contact with the pulp tissue for 21 days, and a large area of regenerated dentin and a long dentin barrier formed along the pulp walls. Thus, observing one case of dentin resorption in the chitosan group does not mean that this substance causes dentin degradation. Yet, this topic is in need of further investigations using different percentages of chitosan.

ZOE paste was used in the present study because of its availability and widespread use. According to the present histological findings, bone resorption was higher in the ZOE group, which may be due to the higher inflammatory response to ZOE compared to chitosan. This result was in line with the findings reported by others [33]. Since overfilling was an exclusion criterion of the present study, this result is unlikely to be due to greater penetration of ZOE into bone.

In the chitosan group, inflammation was significantly less than that in the ZOE group, and this could be due to its antimicrobial properties and biocompatibility. Additionally, histopathological evaluation of periapical tissues in the ZOE group revealed a high level of neutrophils, indicating a severe and acute inflammation in the area [34], which is consistent with the results of Ramos et al, [35] who used propolis paste as an intra-canal medicament after pulpectomy. In histopathological evaluation of periapical tissues of the chitosan group, the macrophage and monocyte levels were higher than in the ZOE group. These cells were found in areas of tissue repair and chronic inflammation [34]. These findings were consistent with the results of Ramos et al, [35] who used a corticosteroid-antibiotic paste as an intracanal medicament after pulpectomy. Based on the histopathological parameters evaluated in dog teeth, tissue integrity of the apical and periapical areas was maintained in the chitosan group. The low penetration of inflammatory cells in the area, the mildly increased PDL width, and the subtle resorption of hard tissues indicate that this material may be used to fill the root canals of primary teeth in pulpectomy. Of course, further studies are required to reach a definitive conclusion. Also, future studies should use other concentrations of chitosan in comparison with different commercially available pastes for this purpose such as calcium-based materials. Additionally, future studies should have a negative control group with no treatment. Since there are many overlaps between the physiological systems of dogs and humans, the present results may be used in future clinical studies on humans.

We did not use primary dog teeth in this study because dogs' primary teeth begin to exfoliate from 3 to 6 months of age. In other words, the primary dentition period is from 0 to 3 months in dogs, and the permanent dentition period starts from 6 months of age. Anesthesia is contra-indicated during the primary dentition period in dogs due to incomplete development of the liver at this age; and only emergency surgeries may be allowed during this period [36-40].

CONCLUSION

In this preliminary animal study, the level of inflammation was significantly lower in 3% chitosan paste group than the control group (teeth filled with ZOE paste). Bone resorption was lower in the chitosan group, while the

lower cementum resorption observed in the chitosan group compared to the ZOE group did not reach statistical significance. PDL widening was milder in the chitosan group compared to the ZOE group. Therefore, 3% chitosan appears to be a biocompatible material. Future human studies are warranted to assess its efficacy.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Babashahi E, Kartalaie MM, Basir L, Rakhshan V. Volumetric Assessment of Root Canal Obturation Using 3% Nano-Chitosan versus Zinc Oxide Eugenol (ZOE) and Iodoform-Calcium Hydroxide (Metapex), in Primary Root Canals Shaped with Rotary versus Manual Methods: A Preliminary In-Vitro Spiral CT Study. Front Dent. 2019 Jan-Feb;16(1):45-54.
2. Imani Z, Imani Z, Basir L, Shayeste M, Abbasi Montazeri E, Rakhshan V. Antibacterial Effects of Chitosan, Formocresol and CMCP as Pulpectomy Medicament on Enterococcusfaecalis, Staphylococcus aureus and Streptococcusmutans. Iran Endod J. 2018 Summer;13(3):342-350.
3. Thomas AM, Chandra S, Chandra S, Pandey RK. Elimination of infection in pulpectomized deciduous teeth: a short-term study using iodoform paste. J Endod. 1994 May;20(5):233-5.
4. Bonow MLM, Guedes Pinto AC, Bammann LL. Antimicrobial activity of drugs used in pulp therapy of deciduous teeth. Braz endod J. 1996;1(1):44-8.
5. Leonardo MR, Silveira FF, Silva LA, Tanomaru Filho M, Utrilla LS. Calcium hydroxide root canal dressing. Histopathological evaluation of periapical repair at different time periods. Braz Dent J. 2002;13(1):17-22.
6. Queiroz AM, Nelson-Filho P, Silva LA, Assed S, Silva RA, Ito IY. Antibacterial activity of root canal filling materials for primary teeth: zinc oxide and eugenol cement, Calen paste thickened with zinc oxide, Sealapex and EndoREZ. Braz Dent J. 2009;20(4):290-6.
7. Gould JM. Root canal therapy for infected primary molar teeth--preliminary report. ASDC J Dent Child. 1972 Jul-Aug;39(4):269-73.
8. Shindova M. Root canal filling materials in primary teeth - review. Folia Med (Plovdiv). 2021 Oct 31;63(5):657-662.
9. Blinder Fuks A, Kapietzki A, M. G. Pulp therapy for the primary dentiton. In: Casamassimo P, Fields H, Mctigue D, A. N, editors. Pediatric dentistry: infancy through adolescen. Maryland Heights, Missouri, USA, : Elsevier; 2013. p. 333-51.
10. Nurko C, Garcia-Godoy F. Evaluation of a calcium hydroxide/iodoform paste (Vitapex) in root canal therapy for primary teeth. J Clin Pediatr Dent. 1999 Summer;23(4):289-94.
11. Wright KJ, Barbosa SV, Araki K, Spångberg LS. In vitro antimicrobial and cytotoxic effects of Kri 1 paste and zinc oxide-eugenol used in primary tooth pulpectomies. Pediatr Dent. 1994 Mar-Apr;16(2):102-6.
12. Fuks AB, Eidelman E, Pauker N. Root fillings with Endoflas in primary teeth: a retrospective study. J Clin Pediatr Dent. 2002 Fall;27(1):41-5.
13. Baumgartner J, Rosenberg P, Hoen M, Lin L. Treatment of endodontic infections, cysts, and flare-ups. Ingle's endodontics. Ontario, Canada: BC Decker; 2009. p. 690-713.
14. Roy PM, Harry P, Cailleux A, Allain P. Dangers of bismuth iodoform paraffin paste. Lancet. 1994 Dec 17;344(8938):1708.
15. Targino AG, Flores MA, dos Santos Junior VE, de Godoy Bené Bezerra F, de Luna Freire H.et al. An innovative approach to treating dental decay in children. A new anti-caries agent. J Mater Sci Mater Med. 2014 Aug;25(8):2041-7.
16. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W. Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules. 2003 Nov-Dec;4(6):1457-65.
17. Sun L, Du Y, Fan L, Chen X, Yang J. Preparation, characterization and antimicrobial activity of quaternized carboxymethyl chitosan and application as pulp-cap. Polymer. 2006 Mar;47(6):1796-804.
18. Ueno H, Yamada H, Tanaka I, Kaba N, Matsuura M, Okumura M.et al. Accelerating effects of chitosan for healing at early phase of experimental open wound in dogs. Biomaterials. 1999 Aug;20(15):1407-14.
19. Matsunaga T, Yanagiguchi K, Yamada S, Ohara N, Ikeda T, Hayashi Y. Chitosan monomer promotes tissue regeneration on dental pulp wounds. J Biomed Mater Res A. 2006 Mar 15;76(4):711-20.
20. Schlueter N, Klimek J, Ganss C. Effect of a chitosan additive to a Sn2+-containing toothpaste on its anti-erosive/anti-abrasive efficacy--a controlled randomised in situ trial. Clin Oral Investig. 2014 Jan;18(1):107-15.
21. Costa EM, Silva S, Pina C, Tavaria FK, Pintado MM. Evaluation and insights into chitosan

antimicrobial activity against anaerobic oral pathogens. Anaerobe. 2012 Jun;18(3):305-9.
22. Peter MG. Applications and environmental aspects of chitin and chitosan. J Macromol Sci, Part A Pure Appl. 1995 Apr;32(4):629-40.
23. Uysal T, Akkurt MD, Amasyali M, Ozcan S, Yagci A, Basak F.et al. Does a chitosan-containing dentifrice prevent demineralization around orthodontic brackets? Angle Orthod. 2011 Mar;81(2):319-25.
24. Hayashi Y, Ohara N, Ganno T, Ishizaki H, Yanagiguchi K. Chitosan-containing gum chewing accelerates antibacterial effect with an increase in salivary secretion. J Dent. 2007 Nov;35(11):871-4.
25. Suzuki S, Masuda Y, Morisaki H, Yamada Y, Kuwata H, Miyazaki T. The study of chitosan-citrate solution as a root canal irrigant: A preliminary report. Oral Hyg Health. 2014;2(142):2332-0702.
26. Jaiswal N, Sinha DJ, Singh UP, Singh K, Jandial UA, Goel S. Evaluation of antibacterial efficacy of Chitosan, Chlorhexidine, Propolis and Sodium hypochlorite on Enterococcus faecalis biofilm : An in vitro study. J Clin Exp Dent. 2017 Sep 1;9(9):e1066-e1074.
27. Camacho-Alonso F, Julián-Belmonte E, Chiva-García F, Martínez-Beneyto Y. Bactericidal Efficacy of Photodynamic Therapy and Chitosan in Root Canals Experimentally Infected with Enterococcus faecalis: An In Vitro Study. Photomed Laser Surg. 2017 Apr;35(4):184-189.
28. Camacho-Alonso F, Julián-Belmonte E, Chiva-García F, Martínez-Beneyto Y. Bactericidal Efficacy of Photodynamic Therapy and Chitosan in Root Canals Experimentally Infected with Enterococcus faecalis: An In Vitro Study. Photomed Laser Surg. 2017 Apr;35(4):184-189.
29. Quest BW. Oral health benefits of a daily dental chew in dogs. J Vet Dent. 2013 Summer;30(2):84-7.
30. Voith VL. Separation anxiety in dogs. The cCompendium on continuing education for the practicing veterinarian (USA). 1985.
31. Riekman GA, el Badrawy HE. Effect of premature loss of primary maxillary incisors on speech. Pediatr Dent. 1985 Jun;7(2):119-22.
32. Silva LA, Leonardo MR, Oliveira DS, Silva RA, Queiroz AM, Hernández PG, Nelson-Filho P. Histopathological evaluation of root canal filling materials for primary teeth. Braz Dent J. 2010 Jan;21(1):38-45.
33. Murata SS, Holland R, Souza Vd, Dezan Junior E, Grossi JA, Percinoto C. Histological analysis of the periapical tissues of dog deciduous teeth after root canal filling with diferent materials. J Appl Oral Sci. 2005 Sep;13(3):318-24.
34. Chen L, Tredget EE, Wu PY, Wu Y. Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing. PLoS One. 2008 Apr 2;3(4):e1886. doi: 10.1371/journal.pone.0001886. Erratum in: PLoS One. 2024 Apr 15;19(4):e0302417.
35. Ramos IF, Biz MT, Paulino N, Scremin A, Della Bona A, Barletta FB.et al. Histopathological analysis of corticosteroid-antibiotic preparation and propolis paste formulation as intracanal medication after pulpectomy: an in vivo study. J Appl Oral Sci. 2012 Feb;20(1):50-6.
36. Center S. A. In: Small Animal Pediatrics: The First 12 Months of Life. Peterson M.E., Kutzler M.A., editors. Elsevier Saunders; 2011. The liver, biliary tract and exocrine pancreas. pp. 368–390.
37. Matthews NS, Mohn TJ, Yang M, Spofford N, Marsh A, Faunt K.et al. Factors associated with anesthetic-related death in dogs and cats in primary care veterinary hospitals. J Am Vet Med Assoc. 2017 Mar 15;250(6):655-665.
38. Brodbelt DC, Pfeiffer DU, Young LE, Wood JL. Results of the confidential enquiry into perioperative small animal fatalities regarding risk factors for anesthetic-related death in dogs. J Am Vet Med Assoc. 2008 Oct 1;233(7):1096-104.
39. Bednarski RM. Dogs and cats. Veterinary anesthesia and analgesia: the fifth edition of Lumb and Jones. 2015 Apr 29:817-26.
40. Short CR. Drug disposition in neonatal animals. J Am Vet Med Assoc. 1984 May 1;184(9):1161-2.