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UNESP - Universidade Estadual Paulista
“Júlio de Mesquita Filho”
Faculdade de Odontologia de Araraquara



Victor Manuel Ochoa Rodríguez

**Physicochemical and biological properties of a new bioceramic root canal
filling material for primary teeth**

Araraquara

2022



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**Physicochemical and biological properties of a new bioceramic root canal
filling material for primary teeth**

Thesis presented to the Dentistry department of
Araraquara, Universidade Estadual Paulista
(Unesp) to obtain the title of Doctor in Dentistry,
in the area of Endodontics

Advisor: Profa. Dra. Gisele Faria

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Physicochemical and biological properties of a new bioceramic root canal filling material for primary teeth

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“Thy Life is a riddle, to bear rapture and sorrow
To listen, to suffer, to entrust unto tomorrow
In one fleeting moment, from the Land doth life flow
Yet in one fleeting moment, for anew it doth grow
In the same fleeting moment
Thou must live
Die
And know”

Answers, Final Fantasy XIV *

* Final Fantasy XIV, composed by Nobuo Uematsu, arranged by Tsutomu Narita, lyrics written by Yaeko Sato and Michael-Christopher Koji Fox, and performed by Susan Calloway.

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ABSTRACT

An ideal root canal filling for primary teeth should not hinder the eruption of permanent successor teeth, but rather, should be resorbed as the primary teeth roots are physiologically resorbed. Bio-C Pulpecto - Bio CP (Angelus, Londrina, Brazil) was recently developed as the first bioceramic filling material for primary teeth. The objectives of **publication 1** were to evaluate radiopacity, setting time, pH, cytocompatibility and the potential for mineralization of Bio-CP compared to Calen thickened with zinc oxide (Calen-ZO) and zinc oxide eugenol (ZOE). The physicochemical properties were evaluated according to ISO 6876. Saos-2 (human osteoblast-like lineage) exposed to material extracts were subjected to methylthiazolyl tetrazolium, neutral red, alkaline phosphatase (ALP) activity and production of mineralized nodule. The results were analyzed using one-way or two-way ANOVA and Tukey or Bonferroni post-tests ($\alpha=0.05$). All materials showed radiopacity greater than 3 mm Al. Bio-CP had a lower pH than Calen-ZO, but higher than ZOE. Calen-ZO and Bio-CP did not set. The ZOE setting time was 110 min. The order of cytocompatibility was Calen-ZO > Bio-CP > ZOE (1:2, 1:4 dilutions) and Calen-ZO > Bio-CP = ZOE (1:12, 1:24 dilutions) and Calen-ZO = Bio - CP > ZOE (dilution 1:32). Bio-CP induced greater ALP activity at 7 days, and greater production of Cal-mineralized proteins, compared to greater numbers of people ($p<0.05$). The objectives of **publication 2** were evaluated for solubility (mass loss), using modified methodology from ISO 6876; filling capacity, volumetric change and presence of voids, by means of micro computed tomography (micro-CT), of Bio-CP in comparison with Calen-ZO and ZOE. In addition, the distribution of elements on the surface of the materials and the crystalline phases of Calen-ZO and Bio-CP were evaluated by energy scattering X-ray scanning electron microscopy (SEM-EDX) and X-ray diffraction (XRD). The materials were placed in polyethylene tubes of 1 or 2 mm in diameter and immersed in water or PBS (Phosphate-Buffered Saline) for 30 days. Data were analyzed by two-way ANOVA and Tukey or Kruskal-Wallis and Dunn ($\alpha=0.05$). Solubility was greater than 7% for all materials; the order was ZOE > Calen-ZO = Bio-CP. Calen-ZO and Bio-CP were more soluble in water than in PBS. Most materials showed higher solubility in 2 mm tubes, both in PBS and in water. Only Calen-ZO and ZOE were analyzed on micro-CT because Bio-CP separated into two phases during scanning. There was no difference in filling capacity between the materials. Calen-ZO had greater volumetric loss and presence of voids than ZOE in water, but there was no difference in PBS. Precipitates on the surface of BioCP and Calen-ZO, corresponding to hydroxyapatite, were drawn after printing in PBS. It was concluded that BioCP presented adequate physicochemical properties, citocompatibility and potential to induce mineralization. All materials showed adequate filling capacity. Bio-CP and Calen-ZO showed lower solubility than ZOE, regardless of immersion medium and tube diameter, except for the 2 mm tube immersed in PBS, and produced hydroxyapatite when immersed in PBS. Micro-CT was not suitable for Bio-CP evaluation. Although Bio-CP has the potential to become a suitable material for filling primary teeth, its composition needs to be revised to achieve chemical stability.

Keywords: Tooth, Deciduous. Endodontics. X-Ray microtomography. Chemical phenomena

Ochoa Rodríguez VM. Propriedades físico-químicas e biológicas de um novo material biocerâmico para obturação de canais radiculares de dentes decíduos [Doctoral Thesis]. Araraquara: Dentistry departmet - São Paulo State University - UNESP; 2022.

RESUMO

Bio-C Pulpecto - Bio CP (Angelus, Londrina, Brasil) foi recentemente desenvolvido como o primeiro material obturador biocerâmico para dentes decíduos. Os objetivos da **publicação 1** foram avaliar radiopacidade, tempo de presa, pH, citocompatibilidade e o potencial para induzir mineralização do Bio-CP em comparação com Calen espessado com óxido de zinco (Calen-ZO) e óxido de zinco e eugenol (ZOE). As propriedades físico-químicas foram avaliadas de acordo com ISO 6876. Saos-2 (linhagem de osteoblastos-*like* de humanos) expostos a extratos dos materiais foram submetidos a ensaios de metiltiazolil tetrazólio, vermelho neutro, atividade de fosfatase alcalina (ALP) e produção de nódulos mineralizados. Os resultados foram analisados empregando ANOVA *one-way* ou *two-way* e pós-testes de Tukey ou Bonferroni ($\alpha=0,05$). Todos os materiais apresentaram radiopacidade superior a 3 mm Al. Bio-CP teve pH mais baixo que Calen-ZO, porém mais elevado que ZOE. Calen-ZO e Bio-CP não tomaram presa. O tempo de presa de ZOE foi de 110 min. A ordem de citocompatibilidade foi Calen-ZO > Bio-CP > ZOE (diluições 1: 2, 1: 4) e Calen-ZO > Bio-CP = ZOE (diluições 1:12, 1:24) e Calen-ZO = Bio- CP > ZOE (diluição 1:32). Bio-CP induziu maior atividade ALP em 7 dias, e maior produção de nódulos mineralizados, em comparação com Calen-ZO ($p < 0,05$). Os objetivos da **publicação 2** foram avaliar a solubilidade (perda de massa), empregando metodologia modificada da ISO 6876; e capacidade de preenchimento, alteração volumétrica e presença de vazios, por meio de microtomografia computadorizada (micro-CT), do Bio-CP em comparação Calen- ZO e ZOE. Além disso, foram avaliadas a distribuição de elementos na superfície dos materiais e as fases cristalinas de Calen-ZO e Bio-CP por microscopia eletrônica de varredura com raio-X por dispersão de energia (SEM-EDX) e difração de raios-X (XRD). Os materiais foram inseridos em tubos de polietileno de 1 ou 2 mm de diâmetro e imersos em água ou PBS (solução salina tamponada com fosfato) por 30 dias. Os dados foram analisados por ANOVA *two-way* e Tukey ou Kruskal-Wallis e Dunn ($\alpha=0,05$). A solubilidade foi superior a 7% para todos os materiais; a ordem foi ZOE > Calen-ZO = Bio-CP. Calen-ZO e Bio-CP foram mais solúveis em água que em PBS. A maioria dos materiais apresentou maior solubilidade em tubos de 2 mm, tanto em PBS quanto em água. Apenas Calen-ZO e ZOE foram analisados em micro-CT porque Bio-CP se separou em duas fases durante o escaneamento. Não houve diferença na capacidade de preenchimento entre os materiais. Calen-ZO teve maior perda volumétrica e presença de vazios do que ZOE em água, mas não houve diferença em PBS. Precipitados na superfície de Bio-CP e Calen-ZO, correspondentes à hidroxiapatita, foram detectados após imersão em PBS. Concluiu-se que Bio-CP apresentou propriedades físico-químicas adequadas, citocompatibilidade e potencial para induzir mineralização. Todos os materiais apresentaram boa capacidade de preenchimento. Bio-CP e Calen-ZO apresentaram solubilidade menor que ZOE, independentemente do meio de imersão e diâmetro do tubo, com exceção do tubo de 2 mm imerso em PBS, e produziram hidroxiapatita quando imersos em PBS. Micro-CT não foi adequada para avaliação do Bio-CP. Embora Bio-CP tenha potencial para se tornar um material adequado para obturação de dentes decíduos, sua composição precisa ser revista para se obter estabilidade química.

Palavras-chave: Dente decíduo. Endodontia. Microtomografia por Raio-X. Fenômenos químicos.

SUMMARY

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1 INTRODUCTION

To achieve success in endodontic therapy, a complete cleaning and shaping, a three-dimensional hermetic sealing throughout the canal and minor discrepancies between the dentinal wall of the root canal and the core filling material is required^{1,2}. The ideal root canal sealer should have the following properties: ease of handling, be radiopaque, provide good adhesion between the canal walls and the core material, establish an hermetic seal, no shrinkage on setting, no staining of the tooth structure, be bacteriostatic or at least not encourage bacterial growth, insoluble in tissue fluids, be biocompatible and soluble in common solvent if a retreatment is required³. On the other hand, the ideal filling material for root canals of primary teeth should not impair the eruption of permanent successor teeth, should not be soluble in water, accompany the physiological resorption of the roots of primary teeth, be quickly reabsorbed when extruded beyond the apex, have antimicrobial properties, adhere to the root canal walls, should not suffer contraction, should be easily removed if necessary, have adequate radiopacity and do not cause teeth color alteration^{4,5}. Many materials have been proposed for filling primary teeth, but none of them meet all the requirements for an ideal filling material^{6,7}.

Zinc oxide and eugenol (ZOE), for a long time, has been widely used as primary teeth filling material^{6,8-12}. However, the deficiencies shown by this material have limited its use. ZOE is genotoxic¹³ and cytotoxic^{13,14}, triggering inflammatory reactions in periapical tissues, particularly when extravasated beyond the radicular apex¹⁴⁻¹⁶. The irritating effect of ZOE has been attributed to eugenol^{6,14}. In addition, ZOE has a low capacity to be phagocytosed and eliminated, leaving particles in the periapical tissues when extravasated beyond the apex and also after the physiological root resorption of the root of primary teeth^{11,12}, taking months or even years to resorb¹⁶⁻²⁰.

Calcium hydroxide-based materials are also commonly used for filling root canals of primary teeth^{6,8,21-24}. Calcium hydroxide [Ca(OH)₂] was introduced in Dentistry by Herman in 1920 for pulp capping²⁵ and, since then, it has been used in Endodontics for different purposes. The main reasons for using Ca(OH)₂ as a root canal filling material for primary teeth are its ability to stimulate mineralization and its antimicrobial effect^{26,27}. These effects occur due to the dissociation of Ca(OH)₂ in calcium and hydroxyl ions²⁸. The antimicrobial effect of Ca(OH)₂ is mainly attributed to its high pH, producing denaturation and damage to DNA and cytoplasmic membranes

in bacterial cells²⁹. In addition, $\text{Ca}(\text{OH})_2$ has the ability to neutralize the LPS produced by gram-negative bacteria^{15,30-34} and neutralize lactic acid secreted by osteoclasts, preventing the loss of mineralized tissue^{26,31}. The effect on mineralization happens because $\text{Ca}(\text{OH})_2$ induces proliferation, migration, osteogenic differentiation of undifferentiated cells and mineralization^{26,35}, through mitogen-activated protein kinases – MAP²⁶.

Calen[®] paste (SS White, Rio de Janeiro, RJ, Brazil), an intracanal medication based on $\text{Ca}(\text{OH})_2$, has antibacterial activity^{27,36,37}, high pH³⁸ and induces the formation of mineralized tissue or nodules³⁹⁻⁴². Studies have shown adequate biocompatibility of the Calen[®] paste compared to other $\text{Ca}(\text{OH})_2$ -based pastes when implanted in subcutaneous tissue of rats⁴³⁻⁴⁵. However, the Calen[®] paste shows permeability to tissue fluids, is soluble in the root canal, has high flow and high viscosity and is, therefore, not suitable for clinical use as root canal filling material for primary teeth²³. On the other hand, Calen[®] paste thickened with zinc oxide (Calen-ZO) has been used as root canal filling material in primary dentition^{24,27,36,37}, showing adequate tissue response^{24,44}, antimicrobial activity³⁶ and radiopacity²³, in the proportions of 1:0.5 and 1:0.65 for Calen[®] paste and ZO respectively²³. The addition of ZO reduces the phagocytosis of the Calen[®] paste, allowing its resorption to accompany the resorption of the roots of primary teeth²⁴. A study has shown that Calen-ZO is more biocompatible than ZOE when used for root canal filling of dog teeth²⁴.

Iodoform-based pastes (KRI), or a combination paste of iodoform and calcium hydroxide are also used to fill the canals of primary teeth⁸ because of its antibacterial effect⁴⁶, healing properties, and ability to be resorbed when in excess⁴⁷. A recent systematic review and meta-analysis showed that the clinical and radiographical success rates of $\text{Ca}(\text{OH})_2$ /iodoform paste are comparable with that of ZOE in primary teeth pulpectomy up to ≥ 18 -month follow-up¹⁰.

New endodontic cements, bioceramics among them, have been developed. Bioceramics, ceramic materials specifically manufactured for use in medicine and dentistry^{48,49}, can be classified into three major groups: almost inert, bioactive and resorbable⁴⁸. They can be composed of alumina and zirconia, bioactive glass, glass ceramics, coatings and composites, hydroxyapatite, calcium phosphates or calcium silicates⁴⁹.

The main characteristics of bioceramic materials for endodontic use (calcium silicate-based bioceramics) are their alkaline pH, antibacterial activity, low contraction,

stability in a biological environment⁴⁹⁻⁵¹, biocompatibility^{49,52-55} and bioactivity⁵²⁻⁵⁵. The hydration of calcium silicate-based cements with tissue fluids⁵⁶ or phosphate-containing solution⁵⁷ forms hydrated calcium silicate gel and $\text{Ca}(\text{OH})_2$. $\text{Ca}(\text{OH})_2$ suffers a chemical reaction with phosphate ions present in tissue fluids, which results in the formation of hydroxyapatite on the material surface^{56,57}. This chemical apatite-forming reaction is thought to be useful for predicting in vivo bioactivity⁵⁷. Hydroxyapatite finally provides a chemical bond with the root canal dentin^{51,58}. In addition, bioceramic cements are hydrophilic, that is, they tolerate and need moisture for the setting reaction^{2,51,59,60,61}. Although calcium silicate-based sealers have suitable physicochemical properties, studies have shown that they have high solubility^{62,63} when evaluated by the ISO 6876⁶⁴ standards.

The biocompatibility and bioactivity of bioceramic sealers for permanent teeth has been studied in different types of cells. EndoSequence BC sealer cement (Brasseler USA, Savannah, GA, USA) showed low cytotoxicity in L929 fibroblasts⁶¹, human gingival fibroblasts² and murine osteoblast precursor cells (IDG- SW3) and promoted osteoblastic differentiation⁵². In human stem cells from the apical papilla, iRoot FS (Innovate BioCreamix Inc.) promoted cell migration and enhanced their osteo/odontogenesis potential, via the Wnt/ β -catenin pathway, without cytotoxicity when compared to ProRoot MTA (Dentsply Maillefer, Tulsa, OK)⁶⁵.

Biocompatibility and in vivo bioactivity of bioceramic materials have also been studied. Sealer Plus BC (Angelus), Bio-C Sealer (MK Life, Porto Alegre, Brazil), NeoMTA Plus (Avalon Biomed Inc., Bradenton, FL, USA), Bio-C Pulpo (Angelus), Biodentine (Sepodont, Saint-Maur-des-Fossés, France) and White MTA (Angelus) showed biocompatibility and bioactive potential when implanted in dorsal subcutaneous sites of Holtzman rats⁶⁶⁻⁶⁸.

Bio-C Pulpecto (Bio-CP; Angelus, Londrina, Paraná, Brazil), the first bioceramic root filling material for primary teeth, is composed of ester glycol salicylate, titanium oxide, calcium tungstate, silicon dioxide, toluene sulphonamide, and calcium silicate. According to the manufacturer, it has high alkalinity (pH 12.7), high radiopacity (9 mm of aluminium scale), and is resorbable, thus allowing simultaneous physiological resorption of the root and the material (Bio-C Pulpecto leaflet, Angelus, Basil, Londrina, Paraná, Brazil). A study showed that the Bio-CP is biocompatible and induced biomineralization in the subcutaneous tissue of rats, in a manner similar to MTA⁶⁹. In addition, a root filling material for primary teeth developed by Angelus, with similar

composition of Bio-CP, but with the name not informed, showed adequate biocompatibility in the subcutaneous tissue of rats, low cytotoxicity in human gingival fibroblast, and satisfactory behaviour regarding the physicochemical properties studied⁷. However, the scientific literature about Bio-CP is still scarce^{7,22,69}.

Endodontic sealers should have low solubility, to prevent microbial and fluid leakage⁷⁰. Furthermore, they should have minimal presence of voids, so it does not affect the obturation quality⁷¹. In case of a primary teeth root canal filling material, even though low solubility is a desirable property, the materials should also be resorbed as the primary teeth roots are physiologically resorbed and should also resorb readily if pressed beyond the apex^{4,5}. Specifically, regarding solubility, they must not have high solubility, to avoid the formation of gaps, nor be insoluble so that they are not retained in the tissue, leading to inflammation, or impairing the eruption of the permanent successor^{72,73}.

Traditionally the solubility of endodontic materials for permanent teeth is evaluated complying the ISO guidelines⁶⁴ which recommends elaborating test specimens of the material with a diameter of 20 ± 1 mm and a height of $1,5 \pm 0,1$ mm. After setting, the test specimens are weighted and immersed in distilled water for 24 hours. Then, the specimens are dried in desiccators and the final weight is compared to the initial weight, determining the loss of mass. However, this model does not reproduce the clinical situation, mainly because tissue fluids are different from water, cement contact time with fluids is not restricted to 24 hours and sealers are immediately placed into contact with tissue fluids and/or blood during root canal treatment⁷⁰. For this reason, to further understand the behavior of these materials, researchers have developed other methodologies to complement the solubility test of root canal sealers^{70,74}.

The methodologies to complement the solubility test of root canal sealers are based on micro computed tomography (micro-CT) to evaluate volumetric change and correlate it with the solubility of the material⁷⁴. The root canals of permanent teeth are filled, and the teeth are immediately placed in the immersion medium⁷⁰. The immersion time varies from 7 to 30 days⁷⁵, and the immersion medium can be distilled water⁷⁵ or phosphate-buffered saline - PBS^{-70,74}. The use of PBS provides greater similarity to clinical situation than water⁷⁶. In this model, the teeth are scanned using micro-CT immediately after filling and after the immersion period⁷⁰. The use of micro-CT provided three-dimensional volumetric analysis (in mm³), allowing correlation of volumetric

change with the solubility⁷². In addition, micro-CT may be used as a method for evaluating morphologic alterations and voids of the materials⁴⁰.

It is important to mention that to assess the solubility of primary teeth filling materials, it is not possible to use the ISO 6876⁶⁴ methodology, since many of them do not set. Thus, an evaluation of the solubility of these materials must be performed by a modified methodology. Researchers have evaluated the solubility of primary teeth filling materials inserted in polyethylene tubes with one end closed²³. However, there is no defined protocol for performing the analysis of the solubility (expressed as loss of mass) of primary teeth filling materials in relation to the diameter of the polyethylene tubes and the immersion medium (water or PBS). In addition, there is no protocol for evaluating volumetric and morphological change in these materials using micro-CT. At first, one could consider using natural primary teeth filled with the studied materials, following methodology models used in permanent teeth⁷⁰. However, as it is very difficult to obtain extracted primary teeth with non-resorbed roots, so alternative methodologies are used. One alternative is using polyethylene tubes²³ to simulate the root canals of primary teeth, because it's easy availability and access in the medical market and handling when prepared as study samples. Another is to evaluate those materials in 3D printed prototyped primary teeth, this is because, they have the potential to be used for educational purposes, endodontic training, and research due to the sample standardization^{77,78}.

Because the scientific literature on Bio-CP is still scarce, and its solubility is not known, studies are necessary to obtain information of its physicochemical and biological properties. In addition, as the absence of defined protocols for evaluating solubility and volumetric change of filling materials used for primary teeth can compromise the scientific impact of the studies, new methodologies must be developed.

4 CONCLUSION

Publication 1

Bio-CP did not set, presented adequate radiopacity and alkaline pH, was cytocompatible and had the potential to induce mineralisation in osteoblast-like human cells (Saos-2).

Publication 2

In general, there was no difference in the solubility of Bio-CP and Calen-ZO, and both had lower solubility than ZOE, regardless of the immersion medium (distilled water or PBS) and tube diameter (1 or 2mm in diameter). Bio-CP and Calen-ZO produced hydroxyapatite after 14 days of immersion in PBS. Micro-CT analysis of volumetric change and voids is not recommended for Bio-CP because it separates in two phases, creating a bias in the comparison of materials. There was no difference in volumetric loss and in the percentage of voids between Calen-ZO and ZOE when immersed in PBS.

Considering the results of publications 1 and 2, Bio-CP has the potential to become a suitable material for root canal filling in primary teeth. Nonetheless, the composition of Bio-CP needs to be revised to achieve better ~~chemical stability and~~ filling capacity without the use of ultrasonics.

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