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**PROGRAMA DE PÓS-GRADUAÇÃO STRICTO SENSU
DOUTORADO EM ODONTOLOGIA**

FABIANO PAIVA VIEIRA

**PROPRIEDADES BIOLÓGICAS E FÍSICO-QUÍMICAS DE
BIOMATERIAIS EXPERIMENTAIS PARA PROTEÇÃO DO
COMPLEXO DENTINO-PULPAR**

Londrina
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Área de Concentração: Dentística

Orientador: Prof. Dr. Sergio da Silva Cava
Co-orientador: Prof. Dr. Alcides Gonini Júnior
Co-orientador: Prof. Dr. Cesar Henrique Zanchi

Londrina
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Área de Concentração: Dentística

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Dedico este trabalho a Deus, meus familiares e amigos.

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RESUMO

O objetivo deste estudo foi avaliar propriedades físicas-químicas e biológicas de cimentos experimentais para capeamento pulpar. Foram desenvolvidos cimentos resinosos de presa dual com diferentes tipos de partículas de carga inorgânica, agregado trióxido mineral (MTA), titanato de cálcio e aluminatos de cálcio (CA) distintos. Estas partículas foram caracterizadas por espectroscopia de infravermelho, espectroscopia por dispersão de energia de raios X, difração de raios X e microscópio eletrônico de varredura. A resistência à tração diametral, potencial hidrogeniônico (pH) e citotoxicidade dos cimentos experimentais foram avaliadas e comparadas com as do MTA. Para a avaliação da cinética de conversão foram realizadas análises em espectroscopia no infravermelho em tempo real (RT-FTIR). Os resultados mostraram o potencial do material experimental em comparação com as principais propriedades físicas e biológicas do MTA, os mais críticos para a triagem inicial de novos materiais. As pastas CA e CLQ (clinker-Fillapex Angelus®) à base de resina apresentaram propriedades semelhantes ou superiores às do MTA.

Palavras-chave: Propriedades físicas e químicas. Propriedades biológicas. Cimentos odontológicos. Capeamento da Polpa Dentária.

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ABSTRACT

Objective: The aim of this study was to evaluate physical-chemical and biological properties of experimental pulp capping cements. **Methods:** Dual-cured resin cements with different types of inorganic filler particles, mineral trioxide aggregate (MTA), calcium titanate and distinct calcium aluminate (CA) were developed. These inorganic filler particles were characterized by infrared spectroscopy, spectroscopy and energy dispersive X-ray, X-ray diffraction and scanning electron microscope. The diametral tensile strength, hydrogen potential (pH) and cytotoxicity of experimental cements were evaluated and compared with the MTA. Real-time degree of conversion was performed in a Fourier transform infrared spectrometer. The results showed the experimental material potential in comparison with the MTA key physical and biological properties, the critical ones to initial screening of new materials. The resin based CA and CLQ (clinker-Fillapex Angelus®) pastes had similar or superior properties to those of MTA.

Key-words: Physical and chemical properties. Biological properties. Dental cements. Dental Pulp Capping.

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1 INTRODUÇÃO

O tecido pulpar é envolto em tecido duro e rodeado por células formadoras deste [1], os odontoblastos, que são células organizadas como uma camada de células em paliçada ao longo da interface entre a polpa dentária e dentina. Eles são responsáveis pela formação da dentina fisiológica primária e da secundária, também participam da manutenção desse tecido duro ao longo da vida útil do dente, sintetizando dentina reacionária em resposta a estímulos adversos ou condições patológicas [2,3].

Algumas condições como a cárie, o trauma ou o procedimento de preparo dentário podem expor a polpa dentária. Uma das alternativas para esta condição é o capeamento pulpar, em que um medicamento é colocado diretamente sobre a polpa exposta (capeamento pulpar direto), ou um material forrador de cavidade é colocado sobre a cárie residual (capeamento pulpar indireto) em uma tentativa de manter a vitalidade pulpar e evitar um tratamento mais extenso exigido pela terapia endodôntica [4]. O potencial para a cura por formação de uma ponte dentinária é bom, desde que a polpa não esteja inflamada [5].

A era da terapia de polpa vital tem sido bastante reforçada com a introdução de vários materiais de capeamento pulpar [6]. Apesar destas alternativas, apenas o hidróxido de cálcio tem uma longa história de indução à formação de pontes de dentina para promover a recuperação pulpar bem sucedida [5]. Porém, a literatura sugere que o agregado trióxido mineral (MTA) é o material de escolha para capeamento pulpar em dentes permanentes em comparação com os materiais usados atualmente [7-11].

Resinas compostas estão surgindo como materiais alternativos para capeamento pulpar, mas a cura é mais lenta, e relativamente pouca pesquisa clínica está disponível para análise [12,5]. Alguns monómeros liberados por estas resinas são citotóxicos e induzem efeitos genotóxicos [13]. A fim de melhorar a biocompatibilidade destes materiais resinosos, foi proposta a utilização de monômeros de elevado peso molecular [14,15]. Além disso, um material resinoso de capeamento pulpar pode ter a vantagem adicional de ligação química com a resina utilizada para a restauração, assim minimizar a ocorrência de falhas no material de capeamento e na interface destes materiais [16], pois alguns autores sugerem que a aplicação do ácido na superfície do agregado trióxido mineral afeta a sua micromorfologia e a força de

ligação deste ao composito [17]. A formulação resinosa também permitiria uma aplicação imediata [18].

Alguns estudos têm demonstrado que um cimento fotopolimerizável à base de agregado trióxido mineral apresenta resultados semelhantes ao MTA[®] (Angelus, Londrina, PR, BR) [18,19]. Outra pesquisa com produtos comerciais, TheraCal[®] (Bisco Inc., Schaumburg, IL, USA), também indicam propriedades semelhantes ou até melhores que o ProRoot MTA[®] (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) [20].

Assim, este estudo propõe a utilização de aluminatos de cálcio [21] como partículas de carga, que podem ser considerados como material potencialmente alternativo ao agregado trióxido mineral [22,23] e materiais resinosos com monómeros de elevado peso molecular [15], a fim de criar materiais com potencial de utilização na proteção do complexo dentino-pulpar. Portanto, as propriedades físico-químicas e biológicas destes serão testadas.

2 REVISÃO DE LITERATURA – CONTEXTUALIZAÇÃO

A polpa dentária é um tecido conjuntivo especializado que, como a maioria dos tecidos humanos, apresenta uma capacidade de regeneração limitada. [24]. A cárie, o trauma ou o procedimento de preparo dentário podem expor esta polpa dentária. A morbidade associada ao tratamento das exposições pulpares pode exigir um tratamento endodôntico, mas um procedimento alternativo para este é o capeamento pulpar, em que um medicamento é colocado diretamente sobre a polpa exposta (capeamento pulpar direto), ou um material forrador de cavidade é colocado sobre cárie residual (capeamento pulpar indireto) em uma tentativa de manter a vitalidade pulpar e evitar um tratamento mais extenso exigido pela terapia endodôntica. As chances de sobrevivência dos dentes são excelentes, se o dente é assintomático e bem selado, mesmo que uma cárie residual permaneça [4]. O potencial para a cura por formação de uma ponte dentinária é bom, desde que a polpa não esteja inflamada [5].

Embora haja muitos produtos, uma recente revisão sistemática concluiu que não existem provas suficientes quanto ao material de capeamento pulpar mais adequado [25]. Alguns materiais de capeamento pulpar utilizados para a proteção do complexo dentino-polpar são o hidróxido de cálcio, cimento de óxido de zinco e eugenol (ZOE), cimento de fosfato de cálcio, ionômero de vidro / ionômero de vidro modificado por resina, agregado trióxido mineral (MTA), Biodentine® (Septodont, St. Maurdes Fossés, France), novo cimento endodôntico (NEC, Shahid Beheshti University, Tehran, Iran), agente resinoso de capeamento pulpar direto com hidróxido de cálcio (Ca-MTYA) dentre outros materiais e métodos [6]. Apesar destas alternativas, apenas o hidróxido de cálcio tem uma longa história de indução à formação de pontes de dentina para promover a recuperação pulpar bem sucedida [5].

Na área da terapia da polpa vital, o agregado trióxido mineral parece ser equivalente e possivelmente superior ao clássico CaOH em termos de capeamento pulpar direto [11]. Os dados disponíveis mostram que a mistura de agregado trióxido mineral com água resulta na formação de hidróxido de cálcio e um ambiente de alto pH. A literatura mostra que o agregado trióxido mineral tem um efeito antibacteriano e antifúngico [26], que é um material biocompatível e não é mutagênico [27].

A literatura sugere que o agregado trióxido mineral é o material de escolha para capeamento pulpar em dentes permanentes em comparação com os materiais usados atualmente [9,10]. As informações atuais sugerem que o agregado trióxido mineral é um material bioativo e possui a habilidade de criar um ambiente ideal para a recuperação pulpar. A partir do momento que o agregado trióxido mineral é colocado em contato direto com os tecidos humanos, sugere-se que o material induz a formação de hidróxido de cálcio que libera íons de cálcio para a fixação e proliferação celular, cria um ambiente antibacteriano pelo seu pH alcalino, modula a produção de citocinas, estimula a diferenciação e migração de células produtoras de tecido mineralizados e forma hidroxiapatita ou apatita carbonatada na superfície do cimento e fornece um selamento biológico [28,9]. O tempo de presa do agregado trióxido mineral é uma das desvantagens deste material. Além disso, a literatura sugere que a presença de diferentes soluções afeta as propriedades físicas do agregado trióxido mineral [26].

Resinas compostas estão surgindo como materiais alternativos para capeamento pulpar, porém pouca pesquisa clínica está disponível para análise [12,5]. Estas resinas odontológicas são biomateriais comumente usados para restaurar esteticamente a estrutura e função dos dentes prejudicados pela cárie, a erosão ou fratura. Monômeros residuais liberados das restaurações de resina, como resultado de processos de polimerização incompleta, podem interagir com os tecidos orais. Alguns monómeros são citotóxicos, induzem efeitos genotóxicos, influem no ciclo celular e a resposta das células do sistema imune inato, inibem as funções dos odontoblastos ou retardam os processos de diferenciação e de mineralização odontogênicos em células derivadas de polpa, incluindo células tronco [13].

A fim de melhorar a biocompatibilidade de materiais resinosos, monómeros de elevado peso molecular, como o dimetacrilato etoxilado bisphenol A glicol (Bis-EMA 30), foram sugeridos pela literatura [14,15]. Assim, em um estudo sobre novos cimentos para capeamento pulpar, a comparação das propriedades físico-químicas e biológicas entre o agregado trióxido mineral, um cimento de ionômero de vidro (CIV) e outros novos cimentos experimentais baseados na combinação sinérgica de materiais existentes (híbrido, pasta e resinoso) foi explorada e o cimento resinoso experimental apresentou desempenho semelhante ou superior aos materiais comerciais e experimentais avaliados [15].

Além disso, um material resinoso de capeamento pulpar pode ter a vantagem adicional de ligação química com a resina utilizada para a restauração, assim minimizar a ocorrência de falhas no material de capeamento e na interface destes materiais [16].

Vários materiais são sugeridos para capeamento pulpar, mas ainda não há um material com as propriedades necessárias para o desempenho ideal. A combinação das propriedades de escolha de diferentes materiais pode permitir o desenvolvimento de novos cimentos com propriedades aprimoradas e melhorar os resultados das atuais estratégias terapêuticas da polpa [24].

A utilização de diferentes partículas de carga, aluminatos de cálcio (CA) e titanatos de cálcio (CaTiO_3), e materiais resinosos com monómeros de elevado peso molecular [15], poderá criar materiais biocompatíveis, compósitos, com potencial para alcalinizar o meio e aumentar os níveis extracelulares de íons Ca^{2+} [22,29,30], propriedades que favorecem a formação de pontes de tecido mineralizado e sua utilização no capeamento pulpar direto.

3 PROPOSIÇÃO

O propósito deste estudo é avaliar determinadas propriedades físico-químicas e biológicas de cimentos experimentais para proteção do complexo dentino-pulpar. Assim, será testada a seguinte hipótese:

A utilização de aluminatos de cálcio (CA) e titanatos de cálcio (CaTiO_3) como partículas de carga associadas a materiais resinosos com monômeros de elevado peso molecular pode resultar em materiais, compósitos, com propriedades físico-químicas e biológicas equivalentes ao MTA.

ARTIGO
BIOLOGICAL AND PHYSICOCHEMICAL PROPERTIES OF EXPERIMENTAL
BIOMATERIALS FOR THE PULP-DENTIN COMPLEX PROTECTION

(A ser submetido ao periódico Dental Materials)

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ABSTRACT

Objective: The aim of this study was to evaluate physical-chemical and biological properties of experimental cements. **Methods:** Dual-cured resin cements with different types of inorganic filler particles, mineral trioxide aggregate (MTA), calcium titanate and distinct calcium aluminate (CA) were developed. These inorganic filler particles were characterized by infrared spectroscopy, spectroscopy and energy dispersive X-ray, X-ray diffraction and scanning electron microscope. The diametral tensile strength, hydrogen potential (pH) and cytotoxicity of experimental cements were evaluated and compared with the MTA. Real-time degree of conversion was performed in a Fourier transform infrared spectrometer. **Results:** The results showed the experimental material potential in comparison with the MTA key physical and biological properties, the critical ones to initial screening of new materials. The resin based CA and CLQ (clinker-Fillapex[®], Angelus, Londrina, PR, BR) pastes had similar or superior properties to those of MTA. **Significance:** the proposed materials have as advantage to be able to bind chemically to the restorative composite resin to form a stronger interface. Another advantage would be the technical simplification of the pulp-dentin complex protection, requiring only two steps and less time.

Key-words: Physical and chemical properties. Biological properties. Dental cements. Pulp-Dentin Complex Protection.

1. Introduction

The dental pulp is a highly vascularized and innervated connective tissue responsible for maintaining the tooth vitality and able to respond to injuries [31] and is encased in hard tissue and surrounded by hard tissue-forming cells [1]. Odontoblasts are post-mitotic cells organized as a layer of palisade cells along the interface between the dental pulp and dentin. They are responsible for the formation of the physiological primary and secondary dentins. They also participate to the maintenance of this hard tissue throughout the life of the tooth by synthesizing reactionary dentin in response to pathological conditions [2].

The consequences of pulp exposure from caries, trauma or tooth preparation misadventure can be severe. The morbidity associated with treating pulp exposures is consequential, often requiring either extraction or endodontic therapy, but an alternative procedure to these options is pulp capping, in which a medicament is placed directly over the exposed pulp (direct pulp cap), or a cavity liner or sealer is placed above residual caries (indirect pulp cap) in an attempt to maintain pulp vitality and avoid the more extensive treatment dictated by extraction or endodontic therapy. The chances for tooth survival are excellent if the tooth is asymptomatic and well sealed, even if residual caries remains [4]. The potential for healing by formation of a dentinal bridge is good, in case the pulp is not inflamed [5].

The era of vital-pulp therapy has been greatly enhanced with the introduction of various pulp capping materials [6]. The highest level of current best evidence has revealed that calcium-enriched mixture cement is a suitable endodontic biomaterial for vital pulp therapy treatments of primary molars as well as mature/immature permanent teeth with reversible/irreversible pulpitis [32]. It appears that mineral trioxide aggregate is the best choice material for pulp capping in permanent teeth compared with currently used materials [9,10,11].

Another option for pulp capping, resin-based composites, may be promising, however more and long-term researches are necessary [12,5]. Studies on the molecular toxicology of substances released by resin-based dental restorative materials clearly support that the majority of these molecules are able to cause cytotoxic and genotoxic effects at concentrations relevant to those released into the oral cavity. These effects include irreversible disturbance of basic cellular functions, such as cell proliferation, enzyme activities, cell morphology, membrane integrity, cell

metabolism and cell viability [33]. To improve the biocompatibility of resinous materials, new monomers with a high molecular weight have been proposed, reducing opportunities for monomer diffusion through dentin and toxicity [14,15]. Furthermore, a resinous capping material may have the additional advantage of chemical bonding with the composite resin used for restoration, minimizing the occurrence of failures at the capping material/restorative material interface [15,16], situation caused by acidic treatment of the mineral trioxide aggregate surface [17]. Resinous formulation can allow light-cure, immediate setting and better working properties [18].

An experimental light-cure mineral trioxide aggregate has been developed to have similar properties to mineral trioxide aggregate, but with better working properties [18,19]. Other research reported that TheraCal[®] (Bisco Inc., Schaumburg, IL, USA), another light-curable MTA-like material for pulp capping, displayed higher calcium-releasing ability and lower solubility than either ProRoot MTA[®] or Dycal[®] (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA). These properties offer major advantages in direct pulp-capping treatments [20,34].

Therefore, this study proposed the use of calcium aluminate (CA) and calcium titanate (CaTiO₃) and resinous materials with high molecular weight monomers to create biocompatible materials [15,22,29,30] with the potential to alkaline environment and to release calcium ions (Ca²⁺), properties that favor the formation of mineralized tissue bridges [9] and their use in the pulp-dentin complex protection. Thus, the biological and physico-chemical properties of these biocompatible materials will be tested.

2. Materials and methods

2.1 Formulation of experimental materials

Calcium aluminate (CA) and calcium titanate (CaTiO₃) are used as the inorganic filler particles to create the experimental groups, they have the potential to increase the biocompatibility of the material developed, to rise the environmental pH and extracellular levels of Ca²⁺ ions [22,29,30], properties that favor the formation of mineralized tissue bridges and the potential outcome of the proposed material.

2.1.1 Synthesis and characterization of inorganic particles

Currently, some methods are employed for the production of the interest CA and calcium titanate crystalline phase, this study used the Pechini method to obtain calcium titanate and combustion for the synthesis of CA, because they have the advantages of being simple synthesis techniques, performed at low temperatures and with a good control of the powders' composition [35,36].

Calcium nitrate tetrahydrated ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) and aluminum nitrate nonahydrated ($\text{Al}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$) and urea ($\text{CO}(\text{NH}_2)_2$) were used to produce CA. Those two reagents and the fuel were obtained from Sigma-Aldrich (St. Louis, USA) and used without any further treatment. The amount of each component required for the chemical reaction to obtain tricalcium aluminate was calculated based on the total of valencies of oxidizing and reducing reagents and fuel [35].

These reagents already weighed on the hot plate was taken onto a heating plate at 90°C and subsequently to the preheated muffle furnace at 400°C [37]. The material obtained in this reaction was heat treated at 800°C or 1200°C during four hours to promote formation of CA crystalline phases [21,35]. A $45\ \mu\text{m}$ size opening particle analysis sieve was used to reduce the filler particle agglomerates obtained at the end of the described process.

Titanium isopropoxide [$\text{Ti}(\text{OC}_3\text{H}_7)_4$] (Vetec, RJ, Brazil), absolute ethyl alcohol (Vetec, RJ, Brazil), anhydrous citric acid ($\text{C}_6\text{H}_8\text{O}_7$) (Synth, SP, Brazil), calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) (Sigma-Aldrich, St. Louis, USA) and ethylene glycol ($\text{C}_2\text{H}_6\text{O}_2$) (Vetec, RJ, Brazil) were used for the production of calcium titanate. Stoichiometric calculations set the required amount of each element in the chemical reaction to obtain calcium titanate, the citric acid/ethylene glycol mass ratio was fixed at 60:40.

Thus, the amount of each reagent was weighed and added in the same sequence of reagents, as described above, in a beaker with constant agitation on a heating plate slowly heated at 100°C to promote citrate polymerization by the polyesterification reaction and to evaporate the solvent, adjusting the viscosity [36,38]. The obtained material, a polymeric resin, was placed in conventional furnace at 300°C for 2 hours, with a heating rate of $1^\circ\text{C}/\text{min}$, promoting the pulverization of the polymeric resin and formation of the precursor powder.

Finally, these material were heat treated at 700°C for seven hours, with a heating rate of 20°C/min, in microwave oven to obtain the filler particles [36]. A 45 µm size opening particle analysis sieve was used to reduce the filler particle agglomerates obtained at the end of the described process.

The characterization of the filler particles was performed by X-ray diffraction analysis (XRD), Fourier transform infra-red (FT-IR) spectroscopy, energy dispersive X-ray (EDX) and scanning electron microscopy [21]. The crystalline phases analysis by X-ray diffraction (XRD) was carried out using the diffractometer Rigaku D/Max2500 PC (Rigaku Corporation, Tokyo, Japan) and Cu K_α radiation at 30 mA and 30 kV, detector rotation between 10° and 80°, with a sampling pitch of 0.02° and scan speed of 2°/min.

Then, the materials were analyzed using a Fourier Transform infrared spectroscopy (FT-IR, Shimadzu Prestige21 Spectrometer, Shimadzu, Japão), with the Happ-Genzel apodization, at a range of 4000 and 600 cm⁻¹, spectral resolution of 4 cm⁻¹ and 10 scans per spectrum. Background noise was removed prior to analysis by background scans.

Elemental constitution of each phase identified was carried out by energy dispersive X-ray (EDX) analysis with a EDX fluorescence spectrometer (Shimadzu, Japão). The filler particles were viewed under a scanning electron microscope (SEM; Model 5400, JEOL, Tokyo, Japan) and particles microstructure, typical particle agglomerates and grain morphology were assessed in back scatter electron mode at 1000X magnification.

2.1.2 Experimental groups

Six groups were proposed, the first one is the standard for comparison, MTA[®] (Angelus, Londrina, PR, BR), and the others were formulated using clinker-Fillapex[®] (Angelus, Londrina, PR, BR), calcium aluminates (CA) and calcium titanate (CaTiO₃) obtained according to the description above without any other treatment. Experimental cements and their respective compositions are presented in Table 1.

Table 1 -. Tested materials, their composition and proportion

Material	Composition	Proportion/ Curing mode
MTA Angelus®	Powder: Portland cement, tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium ironaluminat, bismuth oxide. Liquid: distilled water	Powder / Liquid - 3:1 Chemical
Paste CLQ (CDC-Bio)	Paste 1: 60% clinker-Fillapex Angelus®, 20% Bis-EMA 10, 20% PEG 400 Initiator: 1% DHEPT, 0.8% EDAB, 0.4% CQ Inhibitor: 0.05% butylated hydroxytoluene. Paste 2: 60% Fluoride Ytterbium 20% Bis-EMA 10, 20% Bis-EMA 30 Initiator: 1.5% Benzoyl Peroxide Inhibitor: 0.05% butylated hydroxytoluene	Paste 1 / Paste 2-1: 1 Dual: Chemical and photoactivation
Paste CA 800 (CDC-Bio)	Paste 1: 60% CA (800°C), 20% Bis-EMA 10, 20% PEG 400 Initiator: 1% DHEPT, 0.8% EDAB, 0.4% CQ Inhibitor: 0.05% butylated hydroxytoluene. Paste 2: 60% Fluoride Ytterbium 20% Bis-EMA 10, 20% Bis-EMA 30 Initiator: 1.5% Benzoyl Peroxide Inhibitor: 0.05% butylated hydroxytoluene	Paste 1 / Paste 2-1: 1 Dual: Chemical and photoactivation
Paste CA 1200 (CDC-Bio)	Paste 1: 60% CA (1200°C), 20% Bis-EMA 10, 20% PEG 400 Initiator: 1% DHEPT, 0.8% EDAB, 0.4% CQ Inhibitor: 0.05% butylated hydroxytoluene. Paste 2: 60% Fluoride Ytterbium 20% Bis-EMA 10, 20% Bis-EMA 30 Initiator: 1.5% Benzoyl Peroxide Inhibitor: 0.05% butylated hydroxytoluene	Paste 1 / Paste 2-1: 1 Dual: Chemical and photoactivation
Paste CA (CDC-Bio)	Paste 1: 60% CA (1200°C), 20% Bis-EMA 10, 20% PEG 400 Initiator: 1% DHEPT, 0.8% EDAB, 0.4% CQ Inhibitor: 0.05% butylated hydroxytoluene. Paste 2: 60% CA (1200°C) 20% Bis-EMA 10, 20% Bis-EMA 30 Initiator: 1.5% Benzoyl Peroxide Inhibitor: 0.05% butylated hydroxytoluene	Paste 1 / Paste 2-1: 1 Dual: Chemical and photoactivation
Paste Ti (CDC-Bio)	Paste 1: 60% Calcium titanate, 20% Bis-EMA 10, 20% PEG 400 Initiator: 1% DHEPT, 0.8% EDAB, 0.4% CQ Inhibitor: 0.05% butylated hydroxytoluene. Paste 2: 60% Fluoride Ytterbium 20% Bis-EMA 10, 20% Bis-EMA 30 Initiator: 1.5% Benzoyl Peroxide Inhibitor: 0.05% butylated hydroxytoluene	Paste 1/Paste 2-1: 1 Dual: Chemical and photoactivation

MTA: mineral trioxide aggregate. Bis-EMA: dieterdimethacrylate. PEG 400: poly-ethyleneglycol (400) dimethacrylate. DHEPT: dihidroxietyl-p-toluidine. EDAB: ethyl-4-dimethylamino benzoate. CQ – camphorquinone. CA calcium aluminat.

2.2 Kinetics of Polymerization by RT-FTIR Spectroscopy

The degree of conversion from the experimental materials were evaluated using real-time Fourier transform infrared spectroscopy-attenuated total reflectance (FTIR-ATR) (ZnSe crystal, P IKE Technologies, Madison, WI). A support standardized the distance between the fiber tip of the light curing unit (LED, Radii® Curing Light, SDI, Bayswater, Victoria, Australia) and the sample at 5 mm. The IRSolution software (Shimadzu, Columbia, MD) setup was the Happ-Genzel apodization, at a range of 1750 and 1550 cm^{-1} , resolution of 4 cm^{-1} and mirror speed of 2.8 mm/s and was used in monitoring mode during photoactivation to scan every 1 second. A standardized amount of the sample (0,1g) was manipulated for 60 s and directly dispensed on the ZnSe crystal, then a initial scanning was performed, before, the photoactivation and sample scanning for 60 s. The degree of conversion was calculated considering the intensity of carbon-carbon double-bond stretching vibration (peak height) at 1635 cm^{-1} and using, as an internal standard, the symmetric ring stretching at 1610 cm^{-1} from the polymerized and unpolymerized samples as described in previous literature [39]. The plotted data were analysed for curve fitting and a logistic non-linear regression was performed. These data fitting was used to calculate the polymerization rate (RP (s^{-1})) as the degree of conversion at time t subtracted of degree of conversion at time t^{-1} . The coefficient of determination was greater than 0.97 for CA, CA 800 e CLQ curves, but smaller to the CA1200 (0,919) and Ti (0,918) curves, and the fitting failed for this last one [40].

2.3 Diametral Tensile Strength (DTS) Test

The diametral tensile strength was performed in a universal testing machine (EMIC 2000, Equipamentos e Sistemas de Ensaio LTDA., São José dos Pinhais, PR, Brasil), applying 100kgf load at a speed of 1.0 mm / min. Standard disks ($n=10$, $\text{Ø}=6,0 \pm 0,1\text{mm}$; $h=3,0 \pm 0,1\text{mm}$) were prepared for each experimental group, their borders were gently polished with 600-grit abrasive paper (Norton Abrasivos Brasil, São Paulo, SP, Brazil) and they were stored at 37°C and 100% humidity for 24h and a digital caliper (Mitutoyo 500-144B, Suzano SP, Brazil) was used to measure the disks before the test. The resistance value of diametral tensile strength (σ) was expressed in Mpa.

2.4 Post-Setting pH Changes

The evaluations of hydrogenic potential (pH) were performed at 3, 24, 48, 72 hours, 7 and 14 days, using a digital pH meter (608 Analion PM Plus, Ribeirão Preto, SP, Brazil), calibrated with reference solutions. Standard disks ($n=15$; $\varnothing=4,0 \pm 0,1\text{mm}$; $h=1,0 \pm 0,1\text{mm}$) were prepared for each experimental group. All disks were individually stored in Eppendorf tubes containing 1 ml distilled water and incubated at 37°C during all test period.

2.5 Cytotoxicity

An immortalized cell line, 3T3/NIH mouse fibroblasts, in culture medium (Dulbecco's Modified Eagle's Medium with 4,5g/L Glucose and L-Glutamine – DMEM, Lonza, Walkersville, MD, USA) supplemented with 10% fetal bovine serum and 1% antibiotics (10,000 IU/mL of penicillin G and 10,000 mg/mL of streptomycin; Gibco Laboratories Inc., Grand Island, NY, USA) was used in the cytotoxicity assay. The cells were seeded in culture dishes and maintained in an incubator (37°C , 5% of CO_2) until getting subconfluent.

Thus, a 96-well plate received 2×10^4 cells in 200 μL of culture medium and was incubated with controlled temperature and pressure, in a humid environment at 37°C , 95% air and 5% CO_2 for 24 hours. After this period, there was adhesion of cells at the bottom of the culture plate, forming a cell monolayer which was deposited on the eluates. This was obtained simultaneously by the immersion of the standard disks ($n=6$, $\varnothing=5,5 \pm 0,1\text{mm}$; $h=1,0 \pm 0,1\text{mm}$) of each material individually in Eppendorf micro-tubes containing 1 ml of DMEM culture medium, using the same parameters for incubation at 37°C , 5% CO_2 for 24 hours. These eluates replaced the medium of the test wells and the plate incubated again for the same period under the same conditions (37°C , 5% CO_2 and 24h).

After 24 hours of eluate action on cells, this medium from each well was replaced by 20 μL of 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide (MTT) solution (2mg / ml DMEM) and the plate incubated again for 4 hours to allow its metabolism, to assess cell viability by the MTT assay, which is based on the ability of viable cells to reduce it metabolically, by mitochondrial succinic dehydrogenase enzyme activity, to a blue-purple color formazan crystal that accumulates in the

cytoplasm. After the incubation period, the medium was replaced with 200 μ L of dimethylsulfoxide (DMSO) to resuspend the formazan.

In addition to these wells corresponding to each material tested, was used a positive control (wells with cells without addition of eluates), a negative control (wells without cells, with DMEM only) and compounds used in development of experimental cements alone. This plate was analyzed by spectrophotometry Universal ELISA reader (ELX 800; BIO-TEK Instruments, Winooski, VT, USA), in a wavelength of 570nm where the results were evaluated considering the absorbance values as viability cell indicator.

2.6 Statistical Analysis

Statistical analysis were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, California USA) according to the characteristics of the data and tests, the level of significance of 5% were applied for all tests. Kolmogorov-Smirnov Normality Test was applied to evaluate data's Gaussian distribution. Then One-way analysis of variance and Tukey's test were applied on parametric data and Kruskal-Wallis test e Dunn's Multiple Comparison Test on non-parametric data.

3. Results

Test results are presented in a descriptive way with graphs and tables.

3.1 Characterization of inorganic particles

Particles microstructure, typical particle agglomerates and grain morphology were assessed by the scanning electron micrographs (SEM) in back scatter electron mode at 1000X magnification (Fig. 2). Particle agglomerates and grain size varied, showing irregular morphology.

The powders were analysed by X-ray diffraction (XRD) to identify the present crystalline phases. All diffraction peaks were identified as belonging to the described phase in agreement with the related literature. Figure 3 illustrates the XRD patterns of the samples.

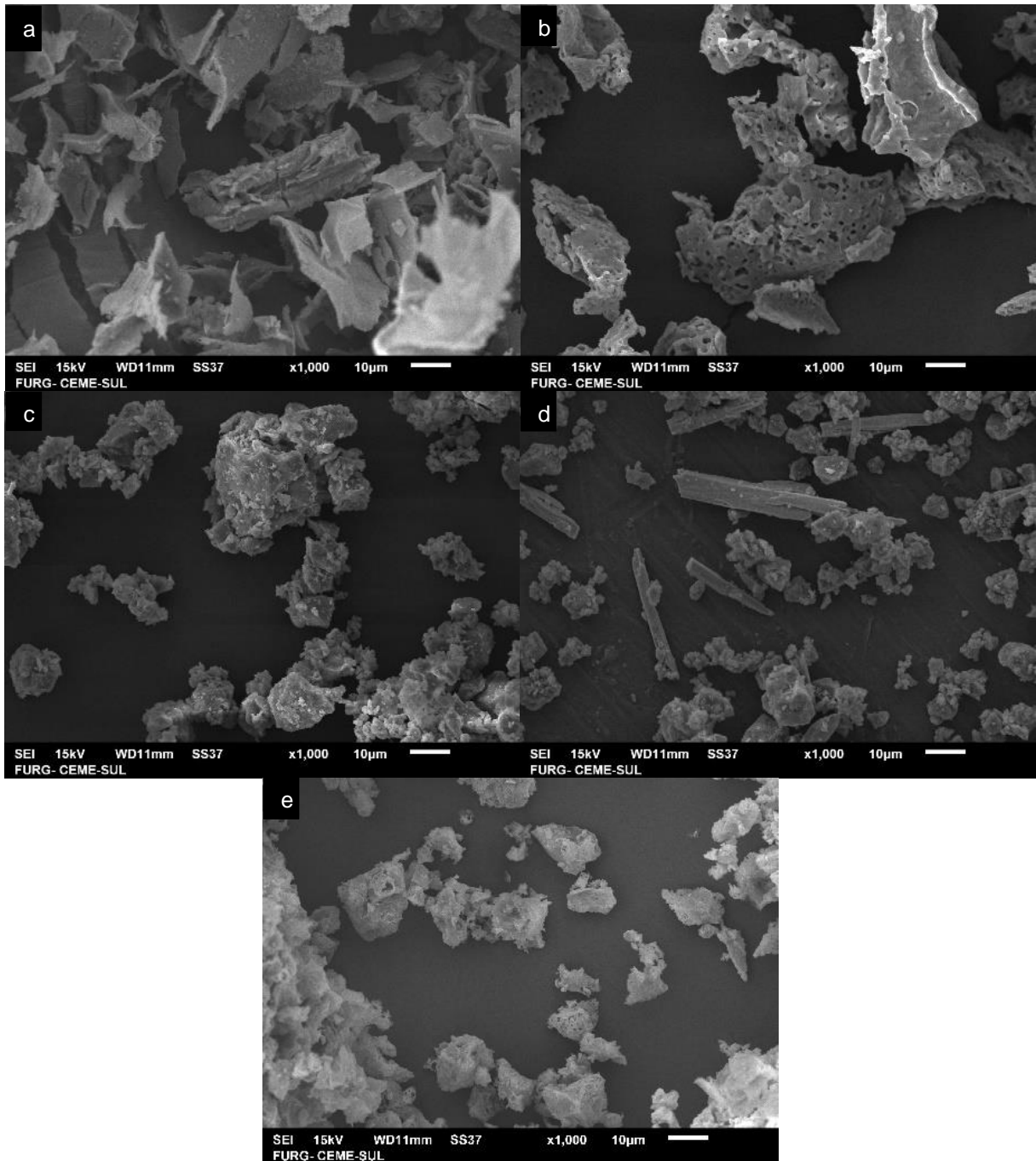


Figure 2 - Scanning electron micrographs (SEM) in back scatter electron mode at 1000X magnification of the stoichiometric as-prepared powders, showing particles microstructure, typical particle agglomerates and grain morphology of (a) CA calcined at 800°C, (b) CA calcined at 1200°C, (c) clinker-Fillapex Angelus®, (d) MTA Angelus®, (e) calcium titanate.

The crystalline phases analysis of CA calcined at 800°C indicated the presence of small crystallites confirmed by the broad bands diffuse and the minor peaks in the X-ray diffraction pattern (Fig. 3a), however it evidenced the formation of crystalline phases, mayenite ($C_{12}A_7$ or $Ca_{12}Al_{14}O_{33}$) and tricalcium aluminate (C_3A or

$\text{Ca}_3\text{Al}_2\text{O}_6$), as well in the CA calcined at 1200°C , although more evidently with higher peaks in the pattern of X-ray diffraction indicating larger grain and particles size (Fig. 3a) [21,41].

The crystalline phase analysis of MTA Angelus® and clinker-Fillapex Angelus® showed tricalcium silicate (C3S, Ca_3SiO_5) indicated by the main peaks at 32.48° and 34.30° and dicalcium silicate (C2S, Ca_2SiO_4) indicated by the main peaks at 32.12° and 32.50° 2θ (Fig. 3b). MTA powder showed peaks at 27.36° , 33.24° and 46.30° 2θ representing bismuth oxide (BO, Bi_2O_3), and peaks at 47.62° 2θ indicating tricalcium aluminate (C3A) (Fig. 3b) [42].

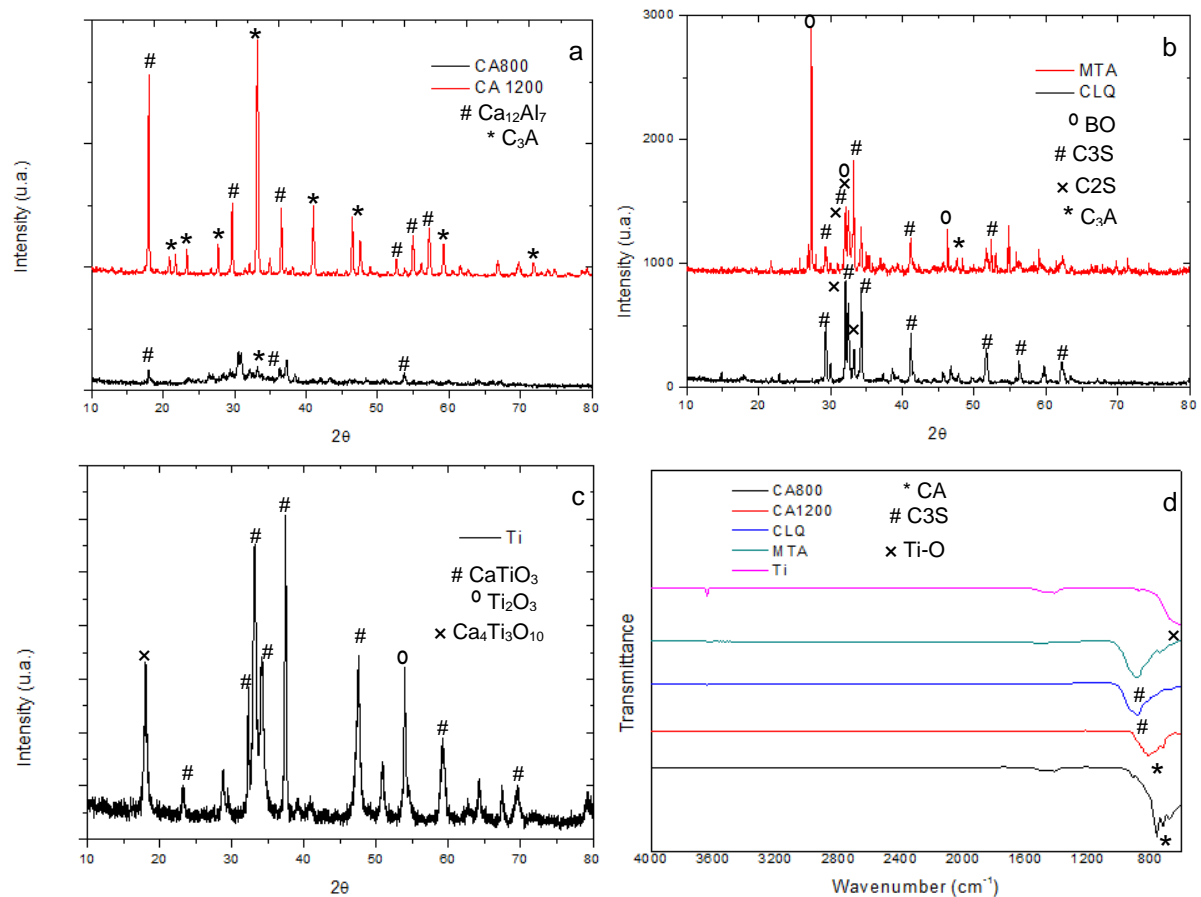


Figure 3 – Characterization of the crystalline phases by X-ray diffraction (XRD) and Fourier Transform infrared spectroscopy (FT-IR), (a) CA calcined at 800°C / 1200°C XRD, (b) clinker-Fillapex Angelus® and MTA Angelus® XRD, (c) calcium titanate XRD and (d) all samples FT-IR.

The crystalline phase analysis of the Ti powder showed calcium titanate indicated by the main peaks at 32.9° , 47° and 59.2° (CaTiO_3 , perovskite). The powder showed peak at 37.36° and 53.74° 2θ representing calcium oxide (CaO) and

titanium oxide (Ti_2O_3), respectively (Fig. 3c) [29,38].

Fourier Transform infrared spectroscopy (FT-IR) analysis of the powders is presented in Figure 3d. The characterization of the crystalline phases of CA calcined at 800°C and 1200°C showed a similar pattern, as well as MTA Angelus® and clinker-Fillapex Angelus®, as expected. Aluminate phases were identified by the absorption peaks in the region $900\text{-}750\text{ cm}^{-1}$, which are attributed to the stretching vibration of the tetrahedral interconnected grid of AlO_4 [21]. Absorption peaks around 875 cm^{-1} indicated tricalcium silicate (C3S) [34]. Strong absorption peaks below 700 cm^{-1} were observed, which are attributed to the stretching vibration of the Ti–O bond [29], representing titanate phases (Fig. 3d).

The elemental analysis of the powders was carried out by energy dispersive X-ray (EDX), then their constitution and the elements' proportion is presented in Table 2.

Table 2 -. Results of the elemental analysis of each filler particles

Material	Ca(%)	Al(%)	Si(%)	Ti(%)	Bi(%)	Fe(%)
MTA	70,3		6,1		21,4	1,5
CA 800	77,5	22,5				
CA 1200	76,5	23,5				
CLQ	91,1		7,9			
TI	76,1			23,5		

Elemental analysis by energy dispersive X-ray (EDX) of MTA Angelus® and the filler particles, CA calcined at 800°C / 1200°C , clinker-Fillapex Angelus® and calcium titanate. The elements proportion Ca (Calcium), Al (Aluminium), Si (Silicon), Ti (Titanate), Bi (Bismuth) and Fe (Iron) are expressed in percentage.

This table shows the predominance of calcium element in the powders, which is expected to be released by the application of the material.

3.2 Kinetics of Polymerization by RT-FTIR

The degree of conversion and rate of polymerization profiles of the experimental cements are presented in Figure 4. These data showed a higher performance of the Paste CLQ and CA 800. The others, Paste CA 1200, Ti and CA, presented their performance in ascending order, respectively, in the degree of conversion (Fig. 4).

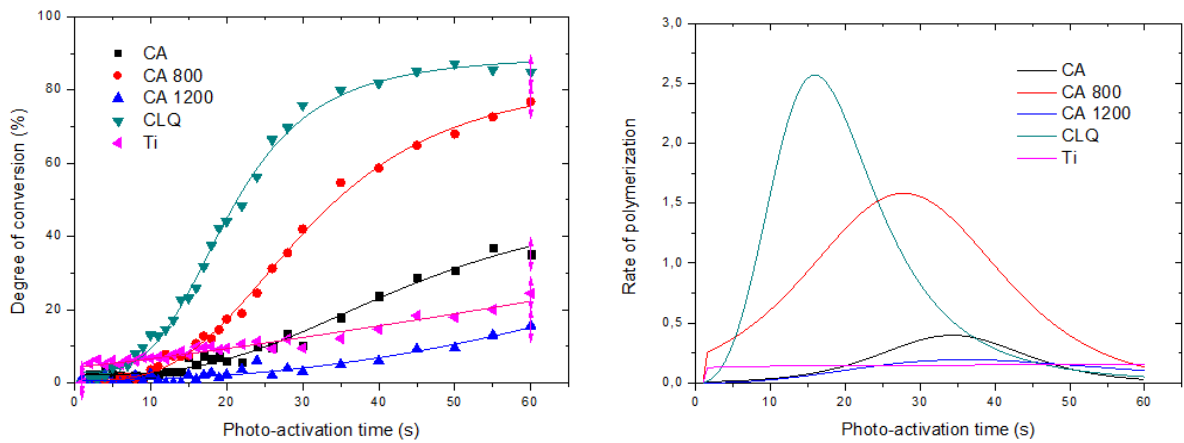


Figure 4 - Degree of conversion and rate of polymerization profiles of the experimental cements.

3.3 Diametral Tensile Strength (DTS) Test

The results of the diametral tensile strength (DTS) test are presented in Figure 5.

There was no statistical difference between the performance of the Pastes that had the best results, Paste CA 800, CA 1200 and CLQ, nor between the performance of the others, Paste CA, Ti and MTA (Table 3).

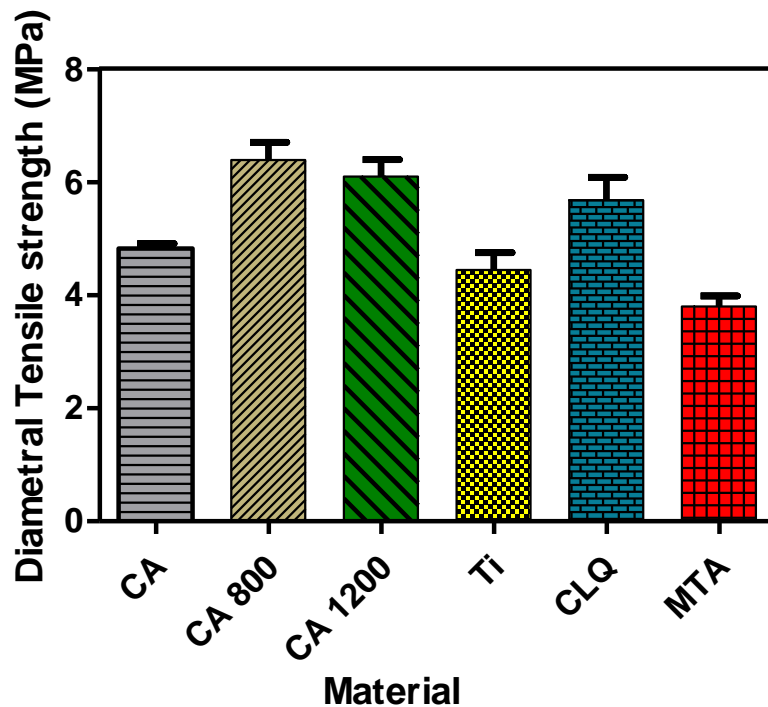


Figure 5 - Results of the diametral tensile strength (DTS) test

Table 3 -. Results of the diametral tensile strength (DTS) test

Material	CA	CA800	CA1200	TI	CLQ	MTA
Diametral Tensile strength (MPa)	4,827 (0,281) ^{ab}	6,397 (0,992) ^c	6,106 (0,933) ^c	4,448 (0,968) ^a	5,684 (1,284) ^{bc}	3,801 (0,588) ^a

One-way analysis of variance and Tukey's test. Different lowercase letters in rows indicate statistically significant difference ($p < 0.05$).

3.4 Post-Setting pH Changes

The results of the pH measurements for the different materials at the time intervals of 3 h, 24 h, 48 h, 72 h, 7 and 14 days are presented in Figure 6. During this period, there was an initial elevation trend in the pH values, but it proved small and still followed by subsequent slight variation or stabilization tendency of the pH values of each material (Fig. 6).

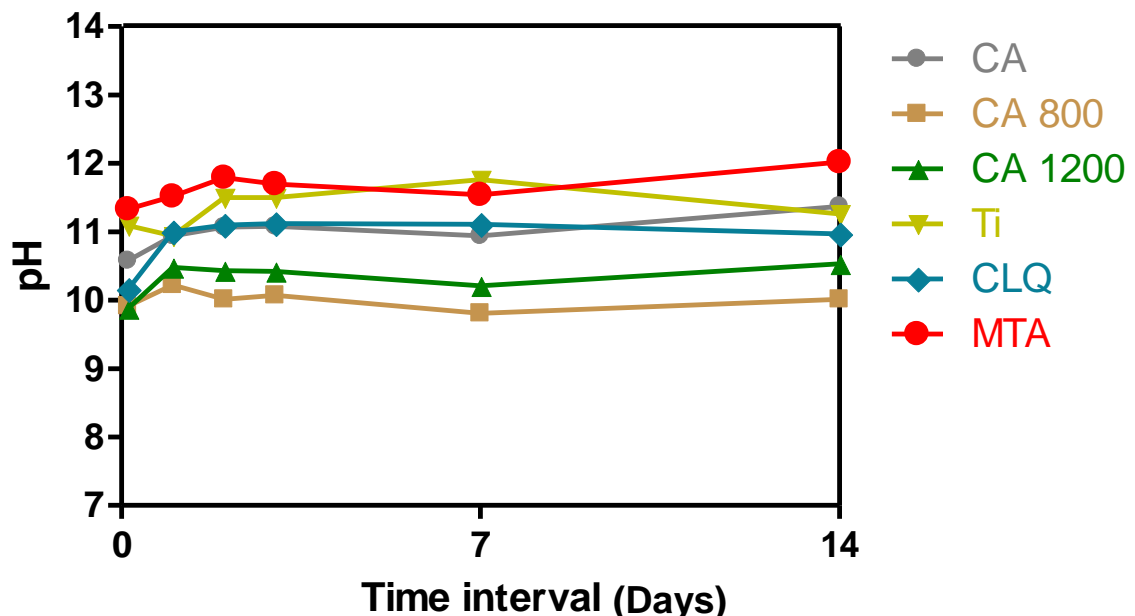


Figure 6 - Results of the pH measurements for the different materials at the time intervals of 3 h, 24 h, 48 h, 72 h, 7 and 14 days

Despite the MTA present the best performance in this test, the evaluation of the range of values of each material in the study period showed a statistically significant increase in pH values only for Pastes CA and CLQ, although it is a small numeric variation (Table 4). The comparison of pH values of the materials in

each time period evaluated showed that at the beginning, in the evaluation of 3h, only the Paste Ti had no statistical difference with the MTA. However, in the last study period, 14 days, Pastes Ti, CLQ and CA also showed no statistically significant difference from the MTA, showing a tendency to the balance of performance between some materials (CA, Ti, CLQ and MTA) in the long time. The Paste CA 800 had the worst performance in this test (Table 4).

Table 4 -. Mean and standard deviations (SD) of pH for the different materials at the time intervals of 3 h, 24 h, 48 h, 72 h, 7 and 14 days.

Time interval	CA	CA 800	CA1200	Ti	CLQ	MTA
3h	10,58 ^{ab} A(0,08)	9,91 ^a A(0,12)	9,88 ^a A(0,10)	11,09 ^{bc} A(0,11)	10,16 ^a A(0,11)	11,33 ^c A(0,08)
24h	10,94 ^{ab} AB(0,21)	10,22 ^a A(0,33)	10,48 ^a A(0,16)	10,94 ^{ab} A(0,21)	11,00 ^{ab} AB(0,07)	11,52 ^b A(0,15)
48h	11,07 ^{ab} AB(0,30)	10,01 ^c A(0,37)	10,43 ^{ac} A(0,21)	11,50 ^{bde} AB(0,08)	11,10 ^{ad} B(0,13)	11,79 ^e A(0,14)
72h	11,08 ^{ab} AB(0,34)	10,07 ^c A(0,37)	10,42 ^{acd} A(0,22)	11,50 ^b AB(0,24)	11,12 ^{bd} B(0,13)	11,70 ^b A(0,16)
7 d	10,94 ^{ab} AB(0,37)	9,80 ^c A(0,60)	10,21 ^{ac} A(0,49)	11,76 ^d B(0,13)	11,11 ^{bd} B(0,22)	11,54 ^d A(0,10)
14 d	11,37 ^{ac} B(0,51)	10,01 ^b A(0,95)	10,53 ^{ab} A(0,74)	11,26 ^{ac} AB(0,66)	10,97 ^{abc} B(0,65)	12,02 ^c A(0,55)

Kruskal-Wallis test e Dunn's Multiple Comparison Test. Different uppercase letters in columns and lowercase letters in rows indicate statistically significant difference ($p < 0.05$).

3.5 Cytotoxicity

Results of the cell viability in the different groups after 24h and 48h in contact with cement eluates are presented in Figure 7.

The analysis of the values in both time periods showed that there were statistical difference between the tested materials, CA, Ti, MTA and the control, presenting the worst results (Table 5).

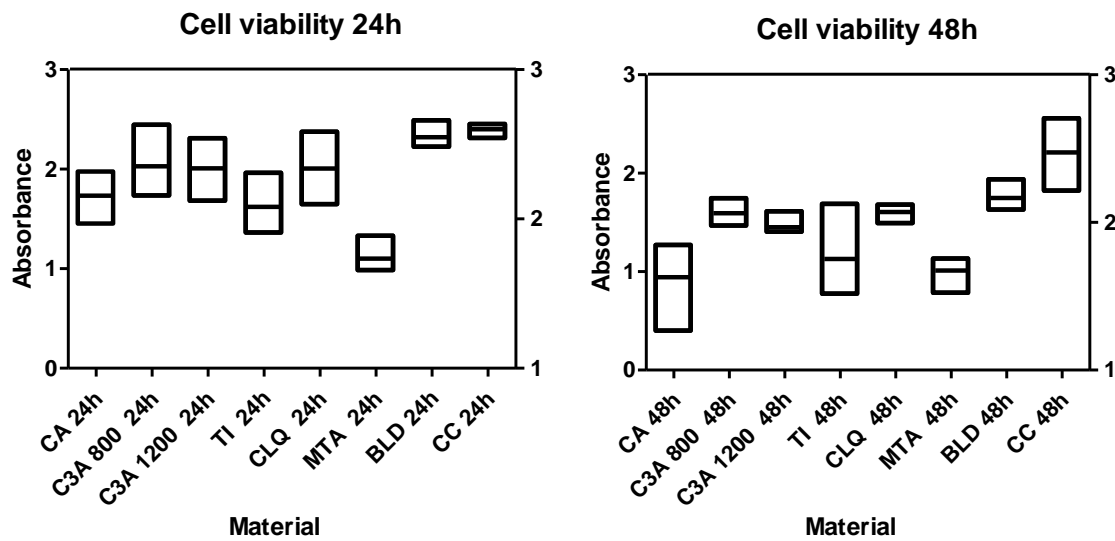


Figure 7 - Results of the cell viability after 24 h and 48 h in contact with cement eluates

Table 5 -. Mean and standard deviation (SD) for cell viability in the different groups after 24 h and 48 h in contact with cement eluates.

Time interval	CA	CA800	CA1200	TI	CLQ	MTA	BLD	CC
24h	1,734 (0,264) ^a	2,030 (0,295) ^{ab}	2,008 (0,261) ^{ab}	1,621 (0,271) ^a	2,007 (0,298) ^{ab}	1,102 (0,147) ^c	2,321 (0,108) ^b	2,401 (0,065) ^b
48h	0,944 (0,338) ^a	1,594 (0,130) ^{ac}	1,450 (0,092) ^{ac}	1,128 (0,374) ^{ab}	1,607 (0,081) ^{ac}	1,011 (0,139) ^{ad}	1,749 (0,131) ^{bc}	2,210 (0,319) ^c

One-way analysis of variance and Tukey's test for 24h / Kruskal-Wallis test e Dunn's Multiple Comparison Test applied for 48h, different letters indicate statistically significant difference ($p < 0.05$).

4. Discussion

Interpretations of the data, their eventual implications and limitations were related to literatura.

The characterization of the inorganic particles showed a mixture of phases as a result of the synthesis process. The preparation of CA powder via solution combustion synthesis using only urea as fuel produced a mixture of CA phases, C₃A and C₁₂A₇ [41, 43] and the additional annealing promoted the degree of crystallinity and grain growth and the formation of CA crystalline phases [35,21].

In the same way, the chemical method employed for the synthesis of CaTiO₃ perovskite generated amorphous carbon powders from residual organic compounds, pulverized citric acid and ethylene glycol. The microwave oven system

used to annealing promotes the rapid phase formation also related with the TiO₂ formation, which is able to absorb partially the microwave radiation [38].

The crystalline phase analysis of MTA Angelus® and clinker-Fillapex Angelus® also showed a mixture of phases, aspect noticed even in the micrographs of the MTA powder. Therefore, this condition was balanced between the samples, although it could have influenced some differences in the test results.

Kinetics of polymerization analysis by RT-FTIR showed the higher performance of more fluid and translucent pastes, CLQ and CA 800, with the higher values of degree of conversion and rate of polymerization, respectively. This can be explained by the factors related to the filler particle. The first factor is the powder density difference. To obtain the same weight of different powders, the volume required for each will depend on their respective densities, so the use of a low-density powder will require greater volume, creating a less fluid and translucent pastes. The other factor is the homogenic distribution of particle size. These factors influence the paste flow capacity, as well as its translucency. The performance in the polymerization kinetics increases in direct proportion to the translucency and paste flow capacity. Thus, the most opaque and less fluid pastes showed the worst performance, Paste CA, CA 1200 and Ti. The ternary photoinitiation system (DHEPT, EDAB and CQ) used contributed to this result.

Furthermore, despite the use of calcium aluminate in different pastes, the results varied widely because of the factors previously described, which is related with the calcination temperature, that promotes difference in particle size and phase distribution, as cited before (Fig. 4).

Despite the low values of the Pastes CA and Ti in the polymerization kinetics, they had a similar performance than the MTA in diametral tensile test, whereas the Pastes CA 1200, CA 800 and CLQ had a superior performance, in accordance with others findings in the literature [15]. These results showed the potencial of the dual cure system by chemical and photoactivation, using a ternary photoinitiation system (DHEPT, EDAB and CQ).

Moreover, the superior mechanical properties of the aluminate is due to the presence of CA phases, mainly C₁₂A₇, which hydrates rapidly, improving the cement setting time properties. Particularly, the Paste CA 800 has small crystallites confirmed by the broad bands diffuse and the minor peaks in the X-ray diffraction pattern, and thus more reactivity, which improves the setting time. Thus, this paste

presents a better mechanical properties than CA 1200, as the effect of augmented annealing temperature, increasing the particle size and grain [21].

The diametral tensile strength was used to test the physical properties of these materials due to their possible future clinical application, considering it probably would suffer significant pressure during restorative procedures [15]. Then, in this aspect, the MTA may be replaced by the proposed materials, because these showed equivalent or better results. Moreover, they still have a potential advantage to be able to bind chemically to the restorative composite resin to form a stronger interface [16]. Another advantage would be the technical simplification of the pulp capping, requiring only two steps and less run time, it is not necessary to wait the setting time. However, the chemical and biological properties are essential to this potential application, so the results related to them will be discussed below.

The results of the pH measurements of all tested materials showed a good potential to alkalize the environment and a tendency of stabilization of the values in relation to the initial ones during the study period. Although the values are slightly higher than other reported, these results were corroborated, especially the described tendency of stabilization of values for a certain period when using resin material [15, 34]. Despite the best numeric performance of the MTA, the equivalent results of Pastes CA and Ti, suggest that both materials are promising for growth factor release from dentin, which has been implicated in signaling events for pulp repair and may favor maintenance of the potential antimicrobial effects for a period of time [15], aspect not considered in this work.

Likewise the chemical properties, the cell viability data in cytotoxicity test showed an equivalent performance of Pastes CA, Ti and MTA, which suggests a potential similar biocompatibility of the materials. This property was increased by the resin component with its lower diffusion characteristics, which had the best result in this test. Furthermore, the good performance of Pastes CA 800, CA 1200 and CLQ, and the literature corroborates these findings, which may in part be a consequence of its high dimensional stability and stable pH post-setting [15].

The opposite performance of Pastes CA, Ti and MTA in cytotoxicity test and in post-setting pH changes suggests an adverse effect of the large increase of pH on the biocompatibility of the material [15,23]. Higher cell viability values in the presence of MTA in 24h evaluation can be found in the literature [44], however as it increases the time of evaluation, the cell viability found values decreased [45]. Thus,

more research are needed to clarify this issue.

Nevertheless, the results showed the experimental material potential in comparison with the MTA key physical and biological properties, the critical ones to initial screening of new materials. The resin based CA and CLQ (clinker-Fillapex Angelus®) pastes had similar or superior properties to those of MTA, corroborating literature findings [15, 22, 23, 34].

5. Conclusion

Calcium aluminate (CA) and calcium titanate (CaTiO_3) used as filler particles in resin with high molecular weight monomers have the potential to create a biomaterial for pulp capping with similar physicochemical and biological properties to those of MTA.

Significance: the proposed materials have as advantage to be able to bind chemically to the restorative composite resin to form a stronger interface. Another advantage would be the technical simplification of the pulp capping, requiring only two steps and less time.

Finally, since the physico-chemical and biological properties have been investigated, it is indicated as future research comparing the radiopacifier potential of CA filler particles with the bismuth oxide. Once the aluminate is cheaper and less toxic.

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