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Development of resin-based bioactive endodontic cements with glycerol salicylate and calcium silicate

Desenvolvimento de cimentos endodônticos bioativos à base de resina com salicilato de glicerol e silicato de cálcio

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ABSTRACT

Introduction: The combination of sol-gel derived calcium silicate particles and glycerol salicylate resins may enhance the pyhisico-chemical properties contribute to the understanding of the interaction between these materials. This study aims to evaluate the physical-chemical properties of resin-based bioactive endodontic cements with glycerol salicylate resins and calcium silicate particles. Materials and methods: Calcium silicate was produced by the sol-gel route, while the resin was produced by mixing 60wt% glycerol salicylate, 30wt% methyl salicylate and 10wt% distilled water. Calcium silicate was added in three different concentrations, 50, 40 and 25% by weight. The cement was tested for setting time, flow, radiopacity and pH. Results: The 50:50 group shows the time of 15min8s while the 75:25 shows the time of 256min13s ($p < 0.05$). The 50:50 group has a lower flow (15.156mm) compared to 60:40 (23.588 mm) and 75:25 (25.396 mm). All radiopacity values were below 3mmAl. All groups showed a pH increase up to 24 hours and the pH value drop was inversely proportional to the amount of calcium silicate. Discussion: Bioactive calcium silicate particles were used in a composite material with a glycerol salicylate resin. Among the tested cements, the combination of 50wt% calcium silicate particles to 50wt% glycerol salicylate resin showed adequate setting time and promoted an increase in water pH. Conclusion: The 50:50 group showed the setting time and the pH, showing that these materials may be able to promote enhanced biological response. The adjustment of flow and radiopacity should be considered for its clinical application.

Keywords: Regenerative endodontics. Dental cements. Resin cements. Glycerol. Biocompatible materials.

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RESUMO

Introdução: A combinação entre partículas de silicato de cálcio e glycerol salicilato tem potencial para melhorar as propriedades físico-químicas de cimentos endodônticos bioativos. O objetivo deste estudo foi avaliar as propriedades físico-químicas de cimentos resinosos contendo glicerol salicilato e silicatos de cálcio. Materiais e métodos: O silicato de cálcio foi produzido pelo método sol-gel, enquanto o líquido foi obtido pela mistura de 60% de salicilato de glicerol, 30% de salicilato de metila e 10% de água destilada em peso. O silicato de cálcio foi adicionado em três diferentes concentrações, sendo elas 50, 40 e 25% em peso. O cimento foi testado em relação ao tempo de presa, escoamento, radiopacidade e pH. Resultados: O grupo 50:50 apresentou tempo de presa de 15min8s enquanto o 75:25 apresentou um tempo de 256min13s ($p < 0,05$). O grupo 50:50 apresentou menor escoamento (15,156mm) em comparação ao 60:40 (23,588mm) e 75:25 (25,396mm). Quanto à radiopacidade, todos obtiveram valores menores que 3mmAl. Todos apresentaram aumento de pH até 24h, e após esse período tiveram quedas inversamente proporcionais a quantidade de silicato de cálcio em cada um. Discussão: Partículas bioativas de silicato de cálcio foram utilizadas em um compósito com base resinosa de glicerol salicilato. Dentre os cimentos utilizados, a combinação de 50:50 entre os componentes tem resultados promissores para a aplicação destes materiais. Conclusão: O grupo 50:50 apresentou adequado tempo de presa e pH, indicando que o material pode manter suas propriedades biológicas. A radiopacidade e o escoamento, no entando, devem ser adequadas para aplicação do material.

Palavras-chave: Endodontia regenerativa. Cimentos dentários. Cimentos de resina. Glicerol. Materiais biocompatíveis.

Introduction

The development of bioactive endodontic cements has been studied as a strategy to enhance the healing of pulp and periapical tissues in regenerative endodontic treatments[1]. These materials can be used for several treatments such as direct and indirect pulp capping, root-end filling, root perforations, and root filling and its ability to stimulate biological response is the main advantage of bioactive endodontic cements when compared to inhert materials[2–4].

The water-based calcium silicate materials are the most used bioactive endodontic cements due to their ability to promote biological sealing of damaged dentin and to stimulate cells to proliferate[5] and differentiate[6] resulting in the formation of regenerative tissue in dentin and pulp. Recently, sol-gel derived calcium silicate particles were purposed as an easy method to produce these particles leading to materials with high reactivity and biocompatibility[7]. These particles are shown to promote *in vitro* cell proliferation and differentiation and emerge as a promising material for the development of bioactive endodontic materials.

Although calcium silicate particles have the desired biological properties for the endodontic application, their handling, their set time and solubility are shown to be the main disadvantages of these materials[1,8]. One strategy to overcome these problems is the development of composite materials combining the low solubility and ease handling of polymeric materials with the bioactivity of ceramic particles. Resin-based endodontic cements are studied using methacrylate, epoxy, and glycerol salicylate resins[9–11]. Due to their increased biocompatibility, glycerol salicylate resins may be an alternative to these materials showing low solubility in recent studies[11,12]. Glycerol salicylates may also contribute to improving the mechanical and setting characteristics[12] of the material when compared to materials that are normally used with water. Also, previous reports showed that these polymers present anti-inflammatory[13,14] properties which may contribute to the development of novel endodontic cements. The combination of sol-gel derived calcium silicate particles and glycerol salicylate resins may be a strategy to enhance the pyhisico-chemical properties and screening these properties may contribute to the understanding of the interaction between these particles and the resin. Thus, the aim of this study is to evaluatethe physical-chemical properties of resin-bases bioactive endodontic cements formulated with glycerol salicylate resins and calcium silicate particles.

Materials and methods

Synthesis of Calcium Silicate Particles

Calcium silicate particles were produced as described previously[7]. Briefly, the sol-gel route was used to produce particles in an acidic environment with nitric acid (HNO3 – Merck KGaA, Darmstadt, Germany), tetraethyl orthosilicate (TEOS – Aldrich Chemical; St Louis, MO, USA) and calcium nitrate (CaNO3 – ÊxodoCientífica, Sumaré, São Paulo, Brazil). After the complete mixture of the reagents, sol was submitted to heat treatment and the resultant particles were grounded and sifted in #400 mesh sieves. The particle size was characterized by laser diffraction (CILAS 1180, France).

Cements formulation

Cements were formulated with the synthesized calcium silicate particles and glycerol salicylate resin blend. The composition was chosen based on previous studies that used similar materials for endodontic applications[11,12]. In this study, the resin blen was formulated with 60wt% glycerol salicylate, 30wt% methyl salicylate and 10wt% distilled water. The formulated resin was mixed with different concentrations of calcium silicate particles, resulting in different resin matrix:inorganic particles proportions. According to this proportion, different groups were formulated: 50:50 (50wt% calcium silicate; 50wt% glycerol resin); 60:40 (40wt% calcium silicate; 60wt% glycerol resin); and 75:25 (25wt% calcium silicate; 75wt% glycerol resin). The resins and the particles were manually incorporated in a glass plate until a homogeneous paste was obtained to produce the samples for the characterization of developed materials.

Setting Time

To assess the setting time of developed cements, the ISO 6876:2012[15] standard was used. The cements were mixed (n = 3) and after 120s were placed in a mold measuring 4mm diameter x 1mm height. With the aid of a Gilmore needle (100g) indentations were made in the cements over time until the no indentation was observed. The time between the mixture and the absence of indentation was recorded as the setting time of the cements.

Flow

The cementsflow was measured according to ISO 6876[15]. The cements mixture (n = 3) was placed between two glass plates (200 mm^2 and 5 mm thickness). A load of 100 grams was applied to the plates and after 180 ± 10 secondsluting agents were photo-activated. The largest and the smallest diameter of the luting agents were measured with a digital caliper. The mean value between the diameters was recorded.

Radiopacity

Radiopacity was tested according to ISO 6876:2012[15]. Specimens (n = 5) measuring 6mm diameter x 1mm height were prepared and submitted to a radiographic analysis in conventional x-ray equipment (Dabi Atlante model Spectro 70X) at 70Kv intensity during 0.8s. Specimens were placed in a phosphor plate with an aluminum step wedge and the images were obtained in a digital system (VistaScan – Durr Dental, Bissingen, Germany). The pixel density in the specimens and in the aluminum step wedge was measured in a standardized area and the gray values of the specimens in an image software (ImageJ, NIH, Maryland, USA) and were converted to mmAl according to the values found in the aluminum step wedge.

pH

Specimens (4mm diameter x 1mm height; n = 3) were immersed in distilled water and the pH of the solution was measured with and digital pHmeter (D-22 Digimed, São Paulo, SP, Brazil). The pH of the solution was recorded before immersion and after 30min, 1h, 1h30min, 2h, 4h, 24h, 72h, 168h e 336h. The average values of pH in each time point was calculated and used to the analysis.

Statistical analysis

The normality of data was assessed with Shapiro-Wilk. Considering the normal distribution of obtained data, one-way ANOVA and Tukey were used to analyze setting time, flow and radiopacity results while two-way ANOVA was used to pH. All analyses were performed at a 5% significance level.

Results

Table 1: Mean and standard deviation of flow and setting time results.

	Flow	Setting time
50:50	15.156mm ± 1.737^{B}	15min8s ± 1min7s^{A}
60:40	23.588mm ± 1.088^{A}	92min46s ± 7min49s^{B}
75:25	25.396mm ± 0.635^{A}	256min13s ± 37min00^{C}

Different uppercase letter indicates a statistically significant difference in each column.

Table 1 shows the results for the flow and the setting time of developed cements. Increased flow was observed 60:40 and 75:25 groups ($p < 0.05$). The 50:50 group showed 15.156mm value, which is lower than the required by ISO 6876[15]. For the setting time, the lower the amount of calcium silicate particles, the higher the setting time. The lowest values were found for 50:50 group with 15min8s of setting time, showing a statistically significant difference to the 60:40 and 75:25 ($p < 0.05$).

Radiopacity results are shown in Figure 1. No statistically significant difference was found between groups in the radiopacity analysis and all groups presented mmAl values that were lower than the ISO 6876 requirements (3mmAl).

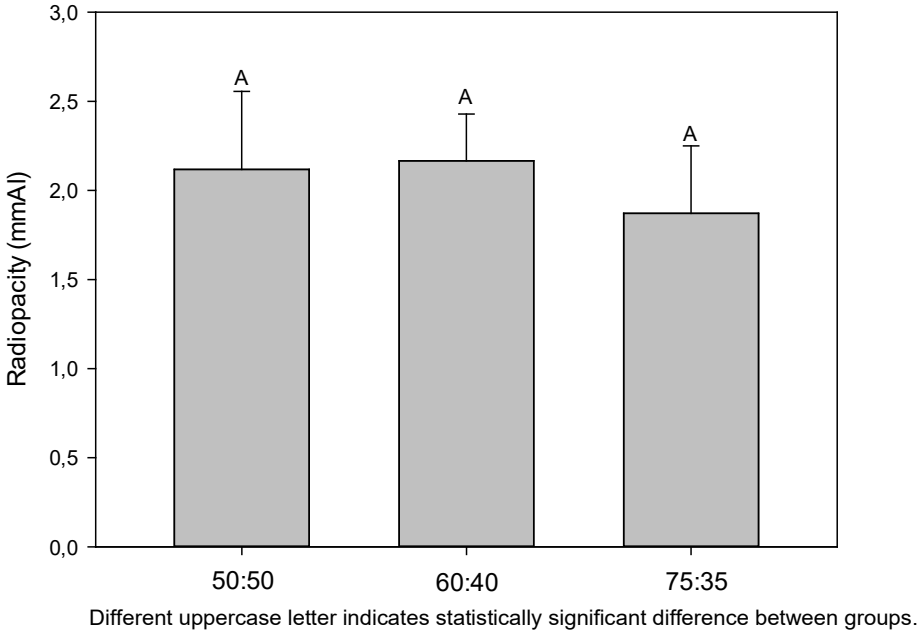

Figure 1: Radiopacity (mmAl) of different cements.

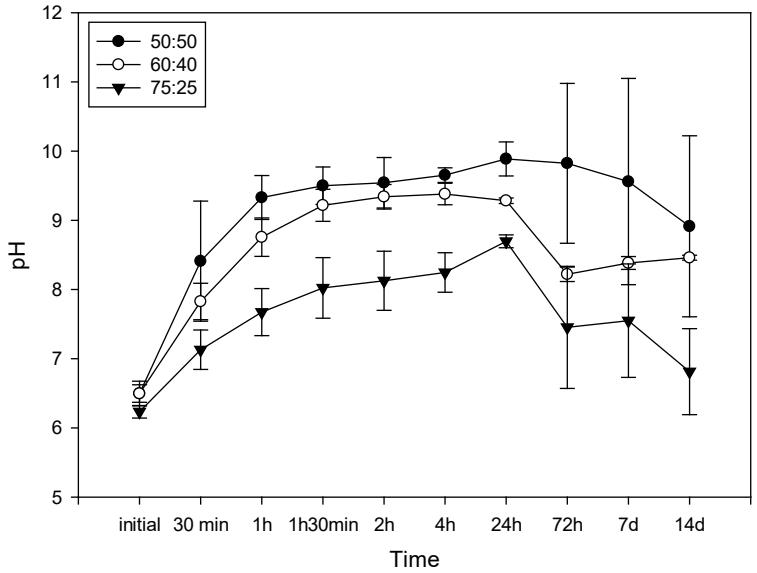

Figure 2: Mean pH values for different cements after immersion in distilled water over different times.

The mean values for pH were shown in Figure 2. The immersion of cements in the distilled water resulted in an increase in pH values for all groups, up to 24h after their immersion. While initial water pH ranged between 6.20 to 6.45, after 24h the 50:50 group increased to 9.80. The 50:50 group presented pH values that were statistically significantly higher than the 60:40 and 75:25 groups. The pH was statistically higher after 24h when compared to the initial values, without a statistical difference to other times ($p > 0.05$).

Discussion

The development of new formulations of endodontic cements has been studied in materials trackingto achieve bioactive properties along with the ability to protect or seal the root canal system[1,5,7,8]. The combination of glycerol salicylate with sol-gel derived calcium silicate particles was tested in the present study for the first time and the pyhisico-chemical properties were tested. Bioactive calcium silicate particles were used in a composite material with a glycerol salicylate resin. Among the tested cements, the combination of 50wt% calcium silicate particles to 50wt% glycerol salicylate resin showed adequate setting time and promoted an increase in water pH.

Calcium silicate particles is used in bioactive endodontic cements such as the mineral trioxide aggregate (MTA)[5,16]. In these materials, the particles were mixed with water and the setting occurs due to hydration of calcium silicate surface[17]. In the present study, the water was substituted for a resin blend produced by the combination of glycerol salicylate and the methyl salicylate, chosen based on previous results[11,12]. These blends are used as an alternative to the other polymers such as methacrylates and epoxy resins that are used in several composite materials in Dentistry but may be not adequate for endodontic applications due to the potential toxicity of when in contact to pulp tissues[2]. The glycerol salicylate blend used in the present studied was shown to present no cytotoxic effect[12] and are studied in other application due to its anti-inflammatory properties based on the inhibition of prostaglandin production[13,14] which may be promising for the application of the developed cements in regenerative procedures.

The content of glycerol salicylate and methyl salicylate was chosen to obtain an adequate viscosity for endodontic applications. As observed in flow analysis, the viscosity of developed materials changed with the addition of particles and the cements with high resin content showed increased flow and were in accordance with the ISO 6876 requirements[15]. The 50:50 group presented values that are lower than the required by ISO 6876 due to the higher amount of calcium silicate particles that leads to an increase in viscosity. Along with the glycerol salicylate and the methyl salicylate, water was incorporated to the resin blend to allow the hydration reaction in calcium silicate particles which may also affect the rheological properties of the developed cements.

The hydration of calcium silicate particles is required for the hardening of the cements and for phase transformation in calcium silicate particles that guarantees its bioactivity[17,18]. In the present study the initial steps of this reaction are especially important for the polymerization of glycerol salicylate resins. In the first steps of hydration of calcium silicate particles, calcium hydroxide (Ca(OH)) is formed due to the dissolution of calcium silicate molecules[19]. The Ca(OH) released from the particles serve as the initiator of the polymerization for glycerol salicylate resins, leading to its hardening and setting over time[12]. The setting time results are explained by these chemical reactions and as observed, the higher the amount of calcium silicate, the lower is the setting time. The higher content of particles to react leads to the formation of a higher amount of Ca(OH), fastening the setting reaction that occurred in 15min for the 50:50 group. On the other hand, when only 25% of calcium silicate was added to the blend (75:25 group), the setting time only occurred after 4h due to the lower availability of particles.

The hydration reaction of calcium silicates is also responsible for the bioactivity of developed cements. It is well known that this reaction is responsible for biological responses when these materials are used for different regenerative procedures in endodontics[3,4,20] that involves the formation of reparative dentinand increased cell response in pulp tissue[21–23]. The increase in pH observed in Figure 2 indicates the formation of Ca(OH) and that the reaction is taking place into the materials. These results were similar to the previous findings for water-based cements that used the same particles[7] and shows the potential of the developed materials to promote modifications to the surrounding environment. The 50:50 group presented increased pH when compared to the groups with a lower amount of calcium silicate, which was expected. Although lower pH was found for the 60:40 and 75:25 groups, no acidic pH was found for any group which shows that glycerol salicylate resins did not release degradation products that may affect the surrounding tissues when cements were used.

One important property for endodontic cements is their radiopacity for the adequate investigation of the cement in clinical applications[24]. According to the ISO 6876[15], endodontic cements must reach 3mmAl to be adequately identified into the root canal system. None of the tested groups reached 3mmAl as seen in Figure 1. This can be explained as neither the calcium silicate particles or the glycerol salicylate resin has radiopaque components in its molecules. Bioactive endodontic cements require the addition of radiopacificants such as ytterbium fluoride[25], calcium tungstate[7], zirconium[5] or bismuth oxide[26] to provide the adequate radiopacity for clinical application of these materials. The formulation used in this study must, in this case, be adjusted with radiopacificant agents for increased mmAl values.

Bioactive endodontic cements are well established as a strategy for regenerative endodontic cements and in the composites produced in the present study show potential to be further investigated for the development of new bioactive endodontic cements. The adjustment of the radiopacity and flow may contribute to the clinical application of these materials. Resin-based cements are known to present increased mechanical properties and stability and the use of biocompatible blends such as the glycerol salicylate. The 50:50 ratio between resin and calcium silicate may be promising after the adjustment os the physical properties and further analysis of mechanical and biological properties of these cements.

Conclusion

Based on the results it can be concluded that the 50:50 group showed promising results considering the setting time and the pH, showing that thesematerials may be able to promote enhanced biological response. The adjustment of flow and radiopacity should be considered for its clinical application.

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