Journal of Dentistry and Oral Care Medicine
Volume 4 | Issue 2
ISSN: 2454-3276

Research Article Open Access

The *In Vitro* Evaluation of Calcium and Bioactive Glass Based Pulp Capping Materials

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Citation: Bostanci B, Gezgin O (2018) The *In Vitro* Evaluation of Calcium and Bioactive Glass Based Pulp Capping. J Dent Oral Care Med 4(2): 208

Abstract

Purpose: Vital pulp preservation in the treatment of deep caries is challenging due to sealing issue. The aim of this study was to compare the physicochemical properties and radiopacity of various light curing pulp capping materials.

Method and Materials: In this study, SEM-EDS analysis and the radiopacity of four different pulp capping materials including ThereCal LC, Biner LC, Calci Plus LC, and Activa-Bioactive were evaluated. Each material was characterized by scanning electron microscopy (SEM) and chemical composition of each material was analyzed by energy dispersive x-ray spectroscopic (EDS) analysis. The disk-shaped specimens (8 mm diameter and 1 mm thickness) were prepared from each material and exposed to a digital x-ray along with an aluminum step wedge for the radiopacity assay. The statistical analysis was performed with ANOVA and Duncan's post-hoc test.

Results: To investigate the chemical composition of the materials, SEM-EDS analysis was performed. The elemental identification showed that contained Ca, Si, O, Al, Na as the major ions. In addition, it was observed bioactive based Calci Plus LC and Activa-Bioactive contain F. TheraeCal had lower radiopacity. No significant differences were observed for radiopacities of the materials.

Conclusion: Bioactive based pulp capping materials offer major advantages in inducing dentin-like tissue formation because of including Ca and F ions highly.

Keywords: Vital pulp therapy; Pulp Capping; Calcium hydroxide; Bioactive glass

Introduction

Vital pulp therapy aims the treatment of reversible pulp injuries that occur during caries or trauma by preserving pulp vitality and function [1]. The placement of a biocompatible material that stimulates the formation of hard tissue to the base of the cavity is defined as pulp capping [2]. It is advised that pulp capping should be carried out usually in young patients based on the high healing ability of pulp tissue [3,4]. Substantially, the success of pulp capping depends on the health status and healing capacity of the remaining pulp tissue. Another significant factor affecting the prognosis of the pulp is capping material [5-8].

The first pulp capping in history was performed by Philip Pfaff in 1796 in order to provide healing of exposed live pulp by putting a small piece of gold [9,10]. Referring to the literature from past to present, several pulp capping materials have been used such as calcium hydroxide, zinc oxide–eugenol cement, polycarboxylate cement, bonding agents, and glass ionomer cement [11]. The material is intended for use in the pulp capping must have numerous properties such as controlling the infection, bonding to dental tissues, preventing microleakage, inducing hard tissue formation and as well as being biocompatible [12,13]. Calcium hydroxide is presented as the gold standard for the evaluation of novel pulp capping materials. Nowadays, calcium hydroxide is still the most current pulp capping material used clinically [14-16].

The use of bioactive glass, a biomaterial, has increased in dentistry recently [17]. The improvement and performance of bioactive glasses developed by Hench *et al.* in 1969 arise from the high biocompatibility of the material [18]. To date, bioactive glass has been incorporated into many materials used in dentistry such as dental sealants, composite resins, regenerative endodontic materials [19-23]. It has been reported that bioactive glass can improve pulp tissue and stimulate mineralization histologically. There is an increasing amount of evidence showing that they have the capacity to serve as inductive materials for hard tissue formation. The pulp is a living tissue with a potency of healing. It has been reported that the failure of pulp capping is due to microleakage and infection. It has been suggested that bioactive glass materials inhibit microleakage by providing the formation of dentin-like tissue [24-26].

Radiopacity is widely acknowledged as a desirable property for all intraoral dental materials, including pulp capping materials [27]. Beyer-Olsen and Orstavik have provided a reproducible standard for comparability in radiographs, and this method is a guide for radiopacity testing procedures [28]. Aluminum step wedge is commonly used to evaluate the radiopacity of dental materials. The use of digital methods that determine gray values has been suggested with the radiograph digitization and the use of specific software to determine the pixel gray values. Afterward, these values are converted into millimeters of aluminum equivalent identifying radiopacity of materials [29-31]. Another significant factor affecting the success of pulp capping treatment is the chemical composition of the materials used [32,33]. It is very important that pulp capping materials contain biocompatible minerals such as calcium, fluoride-induced healing of the pulp. Therefore, the aim of this study was to compare the physicochemical properties and radiopacity of various light curing pulp capping materials. Our two null hypotheses were as follows: (i) There is no difference between the materials regarding radiopacities and (ii) the calcium and bioactive glass-based materials are superior to the others with respect to biological/odontogenic effects.

Materials and Methods

Materials

Four light curing pulp capping materials were selected for this study: TheraCal LC (Bisco Inc, Schaumburg, IL, USA), Biner LC (Meta Biomed Inc, Horsham, PA), Calci Plus LC (Imicryl, Konya, TUR), and Activa Bioactive-Base/Liner (Pulpdent Corporation, Watertown, MA). The components of the materials used in the current study are stated in Table 1.

Material	Components
TheraCal LC	Light-curing, resin-modified calcium silicate filled liner single paste containing CaO, calcium silicate particles (type III Portland cement), Sr glass, fumed silica, barium sulfate, barium zirconate and resin containing Bis-GMA and PEGDMA
Biner LC	Light cure, fluoride releasing, radiopaque cavity liner containing Hydroxy calcium phosphate, UDMA, Photoinitiator, Barium aluminum silicate.
Calci Plus LC	Urethane Dimethacrylate, ULS monomer (Ultra Low Shrinkage Monomer), photoinitiator, stabilizers, ultra Fine Bioactive glass filler, fluoroaluminasilicate glass filler, antibacterial nanocomposite filler.
Activa Bioactive-Base/Liner	Resin-modified glass ionomer, Blend of diurethane and 53.2% other methacrylates with modified polyacrylic acid, Silica amorphous, Sodium fluoride.

Table 1: The components of the materials

Specimen Preparation

Fabricated PTFE/teflon molds with 8 mm diameter and 1 mm thickness were used for the specimen preparation. Each pulp capping material was prepared in accordance with the manufacturer's instructions and placed into the mold then, cured with a light emitting diode light curing unit (VALO, Ultradent Products Inc, South Jordan, UT, USA) with a standard light curing mode for 40 s and thus three standard disk-shaped specimens were obtained for each group. The thickness measurement of each specimen was made with a digital caliper. The specimens were stored at 37 °C for 24 hours in deionized water prior to the tests.

Radiopacity

During the radiographic procedures, an aluminum step wedge with twelve 1 mm incremental steps were used which permitted evaluation of the radiopacity for each pulp capping material with regards to aluminum thickness.

The specimens and the aluminum step wedge were placed on a size-2 phosphor plate (31 × 41 mm-Digora ™ system, Soredex Orion Corporation, Helsinki, Finland) for the comparison of radiopacity of each material. Radiographs were obtained using the X-ray unit with 60 kV, 7 mA and exposure time of 0,2 seconds parameters. The focus distance was set to 30 cm with the test materials. This device maintained the head of the X-ray machine in the same position with the central beam directed at a 90º angle to the surface of the phosphor plate. The phosphor plate exposed to the X-ray beam was scanned using "Digora Optime Radiolasergraphy (RLG)" and images were obtained in digital media. The images acquired were examined using the Digora program and the radiographic values of each specimen were determined based upon the aluminum step wedge. The Digora software for Windows automatically computed the mean grey shade values in the areas which were drawn directly on the images on the monitor. Pixel areas constituting the digital images were standardized for use in each assay. Thus, the gray shade values were obtained. Radiographic values were determined by taking measurements at five different points in each sample and assessing the averages. The radiopacity values in the average of each specimen were converted into equal values to the thickness of the aluminum step wedge (Figure 1).

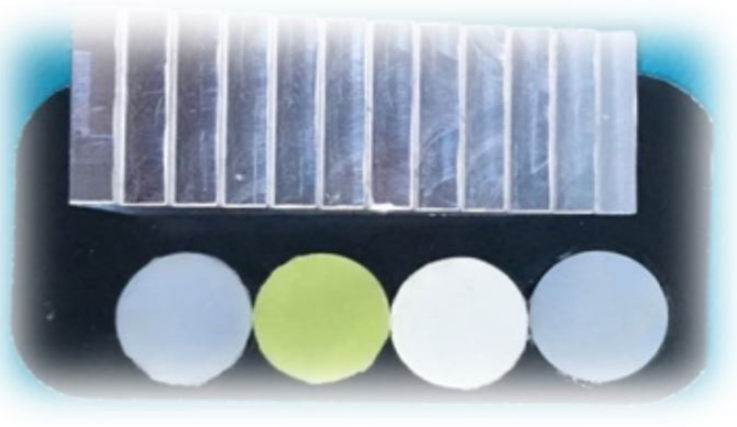

Figure 1: The aluminum step wedge and the specimens

SEM-EDS

SEM-EDS examination of the specimens was performed with SU-1510. The dried samples were first fixed on discs with double-sided carbon adhesive. For the SEM evaluations, the specimens were plated with gold-palladium with a thickness of 50 Angstrom (A °) in Denton Vacuum Desk V Cold Sputter / Etch Unit. The plating process was carried out under a vacuum pressure of 0.05 torr using 30mA current for 60 seconds. Digital images were obtained at different magnification ratios (x500 and x1000) with the beam operating at 10 kV.

Elemental constitution of each material was carried out by EDS analysis. The EDS spectrum was collected at 500x magnification with a collection time of 300 sec. This low magnification was selected to provide an analysis area sufficient to produce the average composition of each sample. The elemental analysis of the sample surface was performed by calculating the percentages of O%, Na%, Ca%, Al%, Si%, Ba%, F% and P% of 6 different spots randomly selected from each surface. All the obtained values were recorded in written form.

Statistical analysis

Statistical analysis was done using commercially available software (SPSS 21.0, SPSS, Chicago, IL, USA). Data were subjected to Shapiro -Wilk test of normality, one way ANOVA and Dunn's Test at the 5% significance level.

Results

Table 2 shows the mean values and the standard deviations of the radiographic density, radiopacity and millimeter equivalents of aluminum for each of the materials. In decreasing order of radiopacity, TheraCal LC was the most radiopaque material, followed by Activa Bioactive Base/Liner, Calci Plus LC, and Biner LC. However, no significant differences were observed for radiopacities of the materials (Figure 2).

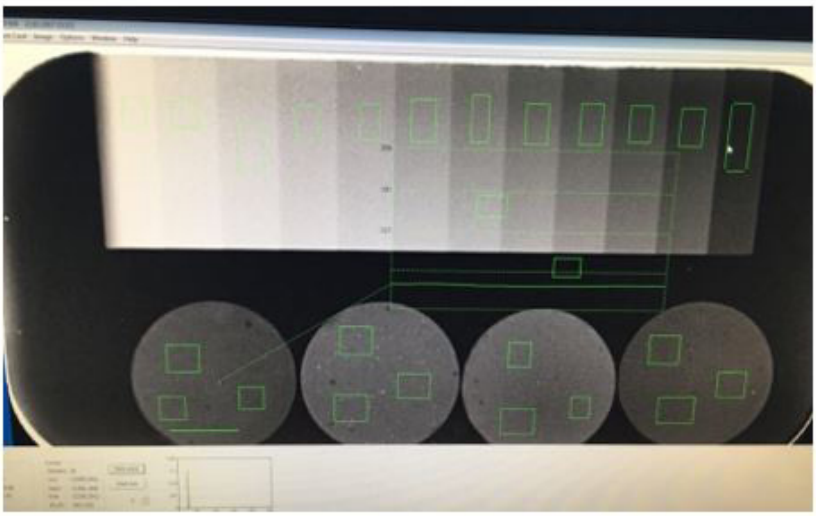

Figure 2: The Digora image

Pulp capping materials	Radiographic density (mean ± SD)	Radiopacity (mmAl)(mean ± SD)
TheraCal LC	51,82±1,44[a]	2,91±0,23[a]
Biner LC	37,74±1,07[b]	1,92±0,12[b]
Calci Plus LC	41,23±1,51[b]	2,01±0,17[b]
Activa Bioactive Base/Liner	46,27±1,33[a,b]	2,21±0,26[a,b]

Table 2: Radiographic densities and radiopacity (mmAl)

To investigate the chemical composition of the materials, SEM-EDS analysis was performed. SEM images of the pulp capping materials investigated in the current study are shown in Figure 3. The elemental identification showed that contained Ca, Si, O, Al, Na as the major ions. In addition, it was observed bioactive based Calci plus LC and Activa-Bioactive Base/Liner contain F (Table 3).

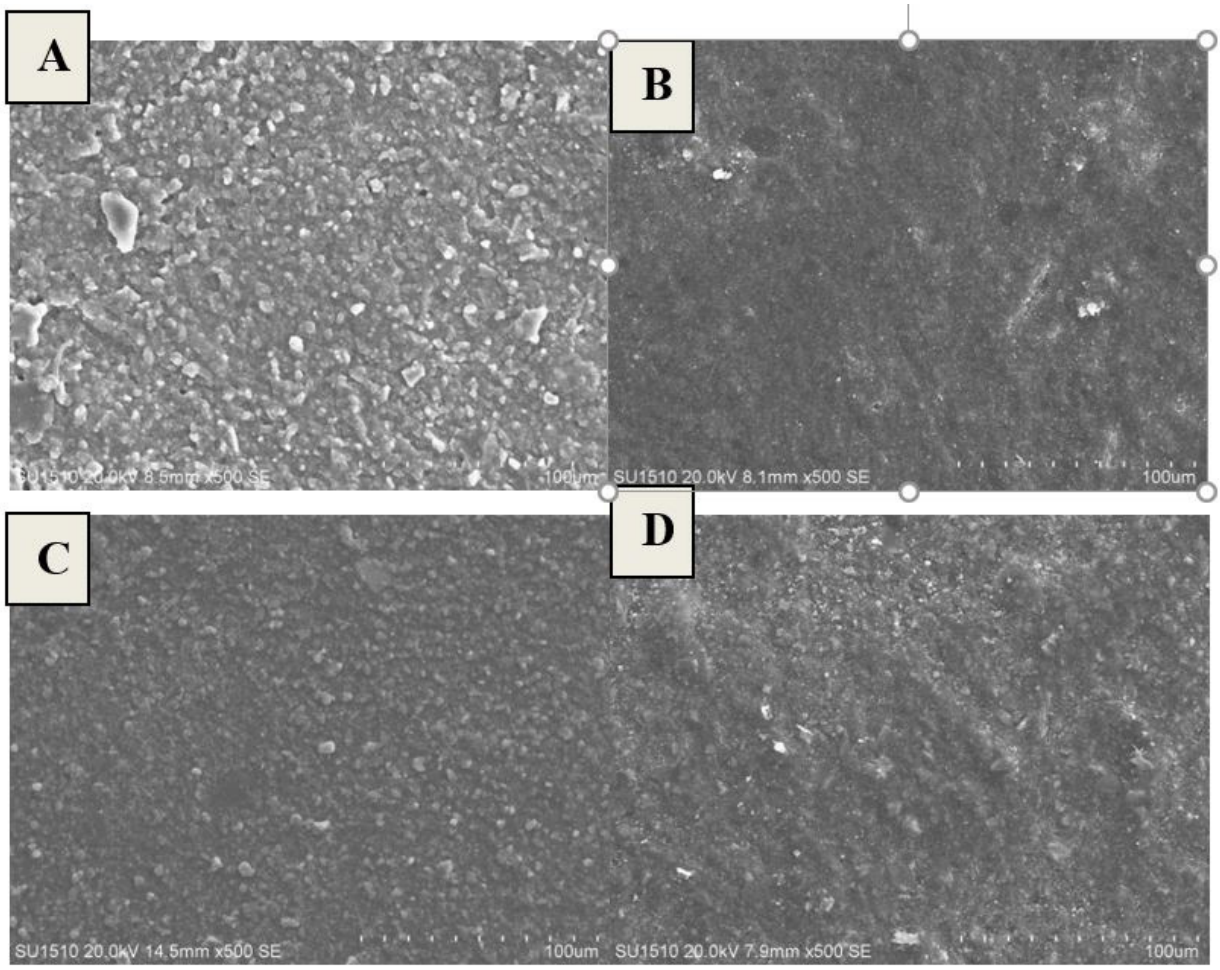

Figure 3: SEM images of the specimens **(A)** TheraCal LC; **(B)** Biner LC; **(C)** Calci Plus LC; **(D)** Activa Bioactive-Base/Liner

Pulp Capping Materials(%)	TheraCal LC	Biner LC	Calci Plus LC	Activa Bioactive-Base/Liner
O	26(±1,22)	30(±9,86)	32(±9,12)	45(±6,18)
Na	0(±0,05)	0(±0,07)	6,3(±3,02)	1,3(±1,01)
Ca	21(±5,36)	0,3(±0,22)	16(±5,04)	3(±1,36)
P	0(±0,02)	0(±0,04)	0,9(±0,22)	0,9(±0,32)
Al	5(±1,85)	8(±3,02)	0(±0,01)	5(±1,32)
Si	28(±7,18)	31(±6,98)	24(±5,03)	25(±9,21)
Ba	5(±3,05)	24(±5,12)	2(±1,12)	17(±3,89)
F	0(±0,06)	1(±0,62)	3(±1,09)	2,9(±1,98)

Table 3: The data of SEM-EDS analysis

Discussion

In this study, we investigated the radiopacity and SEM-EDS analysis of 4 different pulp capping materials. The treatment method of inducing the healing of the pulp or the reparative dentin formation by way of the applying various agent to the pulp is called as pulp capping [11]. The pulpal reactions, becoming prominent by day, have led to come up novel pulp capping materials. The prudential approaches for the improvement of treatment modalities involve the development of new pulp capping materials which are biocompatible, inducing the remineralization [34].

Pulp capping materials should be biocompatible as preserving the contact dental tissue [35]. Calcium hydroxide has been the gold standard for pulp capping [5]. In clinical dentistry, calcium hydroxide is preferred due to its high alkaline and ion release capability. Calcium hydroxide, a chemically corrosive substance, forms a limited coagulation necrosis in the pulp when direct contact with pulp tissue occurs [36,37]. The characteristic of calcium hydroxide has been prompted researchers to develop new materials.

TheraCal (Bisco Inc, Schaumburg, IL, USA) is a light cure, radiopaque, calcium silicate filler resin modified capping material. It has the ability to release calcium to stimulate hard tissue formation and can be used directly under restorations [11,38].

The pulp is a living tissue with the potency of healing. It has been reported that bioactive glass can improve pulp tissue and stimulate mineralization histologically. The use of bioactive glass, a biomaterial, has increased in dentistry recently. To date, bioactive glass has been incorporated into many materials used in dentistry such as dental sealants, composite resins, regenerative endodontic materials [25]. It has been reported that the failure after the pulp capping therapy due to microleakage and pulp infection. It has been suggested that bioactive glass material provides dentin bridge formation, which inhibits microleakage [24]. The studies have conducted that the bioactive glass is more effective pulp capping material than calcium hydroxide [24,25].

In a study comparing calcium hydroxide and bioactive glass material as direct pulp capping material in deciduous teeth, higher levels of inflammation were observed in calcium hydroxide treated samples. Also, internal resorption and abscess formation were observed in calcium hydroxide applied samples, but no internal resorption and abscess formation were observed in bioactive glass applied samples [25].

Marending *et al.* investigated the effects of bioactive glass and calcium hydroxide on the physical properties of dentin. As a result; both of them have been mentioned to have negative effects on the modulus of elasticity and flexure strength, but it has been stated that these effects are further reduced in bioactive glass [39].

The radiopacity of dental materials is valued as one of the basic requirements for accurate diagnosis and follow-up for treated teeth. Aluminum step wedge is commonly used to standardize radiopacity does not provide punctual measurements thus resulting in a subjective method of analysis. The advantage of radiopaque over radiolucent materials is the observation of the radiographic interface between the materials and tooth surfaces. Because of the approximate radiopacity equivalence between dentin and aluminum of the same thickness [40].

Several methods for the determination of the chemical activity of dental materials have been recently recommended. SEM and EDS (used in the present study) are admitted adequate for showing that ion ratios [33]. Chalas *et al*. evaluated the chemical activity of two calcium silicate based pulp capping materials. According to the results of SEM-EDS analysis, both materials have a bioactive potential.

Conclusion

Bioactive glass is considered to be efficient in preventing the progressive childhood caries and the remineralization of initial enamel lesions. In addition, as an alternative material for the pulp capping is ensued. Calcium ions are necessary for the differentiation and mineralization of pulp cells [5]. According to the findings of this study, bioactive based pulp capping materials offer major advantages in inducing dentin-like tissue formation because of including calcium and fluoride ions highly. However, regarding their full mechanisms of action, this requires further research.

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