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An Assessment on pH and Calcium Release of Dycal, Limelite and Biodentine as Cavity Liners

Research Article

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Abstract

Introduction: Indirect pulp treatment is performed on a deep carious tooth that is close to the pulp followed by the placement of a protective layer. The protective layer should promote new reparative dentin.
Aims & Objectives: To compare and assess the pH and calcium release of Dycal, Lime lite and Biodentine.
Materials and Methods: Standardized class 1 cavities were prepared on 36 extracted molars with the excision of pulp. The remaining dentin thickness was maintained from both the coronal and pulpal sides. Nail varnish was applied on all the external surfaces. Samples were divided into 3 equal halves with 12 teeth each. They were treated with an indirect pulp capping procedure by using Dycal, Lime lite and Biodentine. Samples were placed in reverse osmosis water and subjected for pH analysis and assessment of calcium ion release at 3 hours, 24 hours and 48 hours.
Results: The pH analysis after 3 hours showed a significant difference among the 3 groups. There was a significant difference in pH change when compared between Biodentine and Lime lite and no differences between Biodentine and Dycal. The highest calcium ion release was observed with Biodentine at 3, 24, 48 hours. The difference in the amount of calcium released after 24 and 48 hours between the materials Biodentine and Dycal, and Biodentine and Lime lite was statistically significant.
Conclusion: Biodentine demonstrated superior bioactivity than Dycal and Limelite in terms of calcium ion release, and there was no significant variation in pH between the three materials.

Keywords: Biodentine; Calcium Hydroxide; Dycal; Indirect Pulp Capping; Lime lite.

Key message: Cavity Liners Comprised Of Bioactive Materials Could Be Recommended Again For Dentin Regeneration.

Introduction

The primary objective of any pulp capping treatment should be to control bacteria, stop theadvancement of residual caries, stimulate pulp cells to generate new dentin, and provide a biocompatible and long-lasting seal that shields the pulp complex from bacteria and noxious chemicals [1]. Indirect pulp treatment (IPT) is a therapy that is done on a deep carious lesion that is close to the pulp but does not show signs or symptoms of pulpal degeneration [2]. As a protective liner over the affected dentin, materials with bacteriostatic/bactericidal properties have been used. The pace at which calcium ions are released is crucial to the outcome of endodontic and pulp capping procedures [3, 4]. Since 1940, Ca (OH)2 (calcium hydroxide) based products have been used as the gold standard for the procedures. Due to ionic dissociation in the presence of fluids, they release free calcium (Ca2+) and hydroxyl (OH-) ions. Long-term clinical studies have shown that utilizing Ca(OH)2 could promote pulpal repair. However, they have certain drawbacks, including poor sealing ability, long-term disintegration, and difficult manipulation and application [5-8]. Resin-based cavity liners containing calcium hydroxide were invented with excellent physical qualities and handling features to improve the properties [9]. The development of novel biomaterials with improved biocompatibility and seal has influenced attitudes toward IPT in current endodontics, where tissue preservation is a top priority. During the setting phase, hydration causes the release

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of numerous ions [10, 11]. Mineral Trioxide Aggregate (MTA), also releases calcium and hydroxyl ions, is utilized in the pulp capping procedure. On the other hand, clinically, it exhibits some of its drawbacks, such as consistency, manipulation complexity, long setting time, and high material cost, and tooth discolouration [12]. As a solution to this a new tricalcium silicate-based cement called Biodentine (Septodont, France) was developed in 2011. This is being tested for vital pulp therapy and is said to have qualities like MTA. It was invented as a long-term, biocompatible dentin replacement [13]. However, not many studies have demonstrated the ion diffusion rate through both coronal and pulpal sides dentin between these cavity liners. The purpose of the present in-vitro research was to compare and assess the pH and calcium release of Dycal, Limelite (light cure dycal, Pulpdent), and Biodentine.

Materials and Methods

Sample's collection

The current study was approved by the Institutional Ethical Clearance Committee. A total of 36 human maxillary and mandibular molar teeth were collected. All extracted teeth were caries-free or had just a few enamel cavities. The study excluded teeth having extensive caries involving the pulp, any restorations, furcation perforation, and any signs of developmental abnormalities. To remove any callus and soft tissues, ultrasonic scaling was performed, and the teeth were cleaned with tap water, before being placed in a saline solution until they were used again.

Preparation of the samples

All teeth were sectioned at their cementoenamel junction by using a diamond disc. Standardized class I occlusal cavities were prepared on all 36 teeth by using inverted cone diamond bur of 0.2 mm. To obtain a standardized pulp side chamber, pulp tissue was excised with pulp side dentin with the help of the same bur. The distance from the depth of the cavity to the pulp which is the remaining dentin thickness (RDT) was standardized to approximately 1+0.6 mm. It was measured by using an electronic digital caliper (Figure.1: a,b,c) All the samples were treated with 17% Ethylene diamine tetra acetic acid (EDTA) solution to remove the smear layer and rinsed with distilled water. Except for the pulpal cavities, all other lateral surfaces of the resected root dentin and the outer surface of the coronal part were covered by the nail varnish in order the release calcium ions from the dental tissues. While applying the nail varnish, pulpal and coronal cavities were closed with wet cotton pellets to avoid the dehydration of the dentin.The samples were divided into 3 different groups with 12 samples each. Group, I samples were treated by indirect pulp capping procedure by using Dycal (Dentsply, USA) followed by Group II samples with Lime-Lite cavity liner (Pulpdent Corporation, Watertown, USA) and Group III with Biodentine (Septodont®, France) according to the manufacturer's instructions. The cavities from all the group samples were sealed with cavit. The samples were stored separately at a constant room temperature in a beaker with 10 ml of Reverse Osmosis (RO)water with pH 7.

Analysis of pH and calcium ion release

Assessment of pH and the release of calcium ions into soaked water was done by using pH meter (Mettler Toledo) and Atomic Absorption Spectrometry (AAS) (Agilent, AA240) respectively at different time intervals of 3, 24 and 48 hours. Release of calcium ions from all the group samples were measured in parts per million (ppm). Data was collected from both the analysis at all 3-time intervals and subjected for statistical analysis by using one-way analysis of variance (ANOVA) and Tukey's post hoc tests for the multiple comparison between the materials with the SPSS version of 20.

Results

Table.1. ashow the change in the pH of the solutions after the teeth with all three different materials were immersed in RO water at 3 different time intervals. The pH analysis after 3 hours of time interval showed a statistically significant difference among the 3 groups with a p-value of 0.03. When the multiple comparison between different materials was done by using Post Hoc test, there was a significant difference in pH of the solutionbetweenBiodentineand Lime litewhere Lime lite showed the pH of -0.58 ± 0.17 after 3 hours with the p-value <0.05 (Table.1. b).

Statistical analysis of the calcium release showed the significant differences between the 3 groups with the highest calcium ion release seen with Biodentine for 3hours, 24 hours and after 48 hours when compared to other groups (Table.2. a). Multiple comparisons for the distribution of calcium release between the materials showed a significant difference between Biodentineand Lime lite and between Dycaland Lime lite at 3 hours (p <0.05). However, there was a significant difference in the amount of calcium released after 24 hours and 48 hours between the materials such as, Biodentine and Dycal and Biodentineand Lime lite (Table.2.b).

Figure 1(a, b, c). Preparation of the cavity from both coronal & pulpal side to obtain standardized remaining dentin thickness (RDT) and measured by using electronic digital calliper.

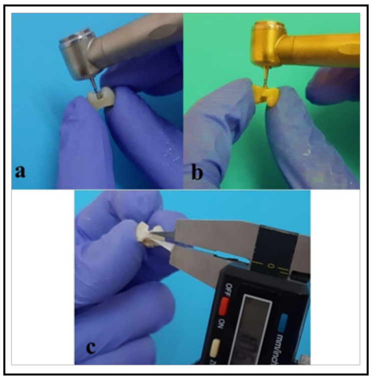

Table.1. a: Effect of time and materials to the pH of RO water
b. Multiple comparisons of materials by using Post Hoc test with the p value *<0. 05.

a. Materials		**pH value at specific time intervals**		
		3 h	24 h	48 h
Dycal		7.30 ± 0.25	7.48 ± 0.24	7.57 ± 0.25
Lime lite		7.45 ±0.16	7.65 ± 0.11	7.72 ± 0.09
Biodentine		6.86 ± 0.20	7.29 ± 0.20	7.54 ± 0.05
p value		0.03	0.08	0.39
b. Comparison of Materials		**3 h**	**24 h**	**48h**
Biodentine	Dycal	-0.43 ± 0.17	-0.19 ± 0.13	-0.02 ± 0.12
	Lime lite	-0.58 ± 0.17*	-0.36 ± 0.13	-0.17 ± 0.12
Dycal	Lime lite	-0.15 ± 0.17	-0.17 ± 0.13	-0.15 ± 0.12

Table 2. a: Calcium release data (measured as ppm) for different materials at varied time.
b. Multiple comparisons of materials for calcium release using Post hoc test with the p value **<0.001, *<0.05.

a. Materials		**pH value at specific time intervals**		
		3 h	24 h	48 h
Dycal		3.83 ± 0.19	6.43 ± 0.21	7.03 ± 0.15
Lime lite		0.50 ± 0.07	1.02 ± 0.10	1.73 ± 0.20
Biodentine		74.47 ± 1.91	340.76 ± 12.88	351.80 ± 10.23
p value		<0.001	<0.001	<0.001
b. Comparison of Materials		**3 h**	**24 h**	**48 h**
Biodentine	Dycal	70.63 ± 0.90**	334.33 ± 6.07**	344.76 ± 4.82**
	Lime lite	73.96 ± 0.90**	339.74 ± 6.07**	350.06 ± 4.82**
Dycal	Lime lite	3.32 ± 0.90*	5.40 ± 6.07	5.30 ± 4.82

Discussion

The most important factor for any successful pulp capping treatment is the appropriate case selection with the procedure involving a placement of protective material on a thin partition of RDT followed by sealing with a good restoration [14, 15]. As a result, it's becoming increasingly popular to use agents that release calcium and hydroxyl ions, as calcium's activity aids in the mineralization of hard tissue and tissue regeneration [16, 17]. When considering the bio interactive features of these ions, the capacity of the materials to release ions may be essential. However, as the pH rises and the concentration of calcium ions rises, the solution becomes more supersaturated with apatite, promoting the formation of a carbonated apatite coating layer on the cement surface [18]. Overall, calcium release is associated with improved antimicrobial performance, whereas alkalinity affects cytotoxicity and triggers fibroblast growth [19, 20]. Calcium ions also boost the proliferation of human dental pulp cells in a dose-dependent way and promote the activity of pyrophosphatase, which helps to maintain dentine [21].

Despite numerous studies analysing pH and calcium ion release from various materials and performing solubility testing in distilled water/deionized water, as well as in phosphate containing Phosphate Buffered Saline (PBS) buffer and Butyric acid solution as a medium [22], there has been no research on pH solution analysis or calcium ion release from RO water. Furthermore, due to a varied chelation impact, different pH buffers and different types of acids have different effects on calcium ion release [23].

Ion diffusion from calcium hydroxide through root dentin has already been proven in previous studies [24, 25]. Some of them have reported the ion diffusion from mid root dentin [26]. In our study, we have assessed ion diffusion through both the coronal and pulp sides of the dentin while preserving the RDT to improve permeability [5, 27]. To assure the patency of the dentinal tubules, the RDT's dentin surfaces were treated with EDTA to eliminate the smear layer, which includes bacteria, and necrotic tissue, which may act as substrates for bacterial survival, as well as any likely source of calcium ions [28]. It is safe to assume there was no other supply of calcium because the soaked solution was RO water, which contains no minerals [29]. The $Ca(OH)_2$ of the cavity liners produced Ca^{2+} ions in the RO water.

The present study investigated the variations in pH and release of calcium ions from Dycal, Lime lite and Biodentine as cavity liners. To compare with alternative materials, Dycal, a self-setting calcium hydroxide-based cavity liner, was chosen. It comprises a plasticizer (sulphonamide) and a setting activator (butylene glycol disalicilate). Calcium and hydroxyl ions are provided by the catalytic paste [30]. When compared to Biodentine and MTA Angelus, Dycal exhibited a lower rate of calcium and hydroxyl ion release over 28 days [31], which was comparable to the results of the current study, where Dycal showed less calcium release as compared to Lime lite and Biodentine. Other research findings, on the other hand, have revealed that Dycal causes calcium release [32].

Lime lite, a light curing material, containing hydroxyapatite in a urethane dimethacrylate resin showed a slight change in the pH compared to Biodentine and Dycal only after 3 hours. This is in agreement with the other study findings, which found that light cured calcium hydroxide cements released more calcium ions for up to 21 days than Dycal [33]. The resin portion of the light cured calcium hydroxide cement can enhance calcium and hydroxyl ion release within the wet area on the tooth dentin and pulp, favouring interaction with the hydrophilic tooth dentin [34]. On the contrary, calcium silicate materials such as MTA Plus gel and Biodentine showed the highest calcium release and better alkalinity compared to Lime lite [35].

Biodentine, has been referred to as a bioactive dentin alternative, after being immersed in phosphate solution, it forms apatite, indicating that it is bioactive [36]. The current study results revealed that, Biodentinereleased more calcium ion than Dycal and Lime lite. This is in line with studies that demonstrated Biodentine to have a higher calcium release rate with the value of 15.67 (±0.66) than MTA after 48 hours [11] and a higher calcium release rate when compared to other Bio-C Pulpo and TotalFill products root repair material [19]. The structure and existence of size of mineral particles in Biodentine, together play an important role in its solubility and water resorption, which explains the release of ions. The calcium phosphate particles generated from the material have a diameter of less than one micron, resulting in a more compact surface layer. That could be the reason for Biodentine's higher calcium ion release [4]. Furthermore, the presence of calcium carbonate was shown to play a significant role in the hydration reaction [37]. General mechanism involves, Biodentine react with water shortly after mixing to produce a high-pH solution comprising silicate, calcium & hydroxyl ions, which raises the alkalinity of the medium [38]. Other investigations have shown that calcium silicate cements can generate hydroxyapatite crystals after contact with saliva and other phosphate-containing body fluids. However, it is unknown how much these crystal forms affect solubility, and the effects of ions present on the dissolution process of these cements have yet to be investigated [39, 40].

Even though the manufacturer states that the materials used in the study have a high pH of nearly 12, the current investigation indicated that the maximum pH value seen with Dycal and Lime lite after all three-time intervals was over 7. Biodentine had a pH of 6.8 after 3 hours, which is slightly acidic, and these materials were shown to better alkalize the soaking medium after 24 and 48 hours, with a pH of > 7, which is alkaline. These obtained values could be due to the possible factors which have been demonstrated in various studies such as volume and the exposed surface area of the material to the surrounding medium.In those experiment's greater pH and calcium ion release were consistently observed using Pro Root MTA with a 50.24 mm^2 exposed surface area [41-43] and Biodentine with a 140.74 mm^2 [44]. Calcium ion release was also reduced in trials that employed a smaller exposed surface area with 0.79 mm^2. The current study, however, did not include an assessment based on these criteria. Another reason could be the RO water, which had a PH of 7 and was employed as a soaking medium. It has been proven that when it is exposed to air, it lowers to an acidic pH range of 5.5 or below within an hour as it absorbs carbon dioxide from the surrounding air, necessitating the addition of calcium and other minerals to alkalize it [45-47]. More research utilising RO water are required to investigate how it influences pH fluctuations and calcium ion release from the materials.

In contrast to the current investigation, an increase in calcium ion release was seen in a pH 4.4 solution. It could be owing to the cement's higher solubility in an acidic environment. As a result, increasing porosity with restorative materials. When applied to a clinical situation, this would imply the need for an alkaline medication to be implanted. In the clinical instance, it also means that a high alkaline medium affects calcium ion release, and that complete irrigation of the root canal space is a must to balance the pH of the root canal [48].

Although the current invitro investigation proved ion diffusion via the coronal and pulp side dentin by using reverse osmosis water, it was unable to replicate the normal oral environment with inflamed pulp as it is linked with an increase in pulpal pressure and temperature [49]. More *in vivo* research is required to assess these bioactive materials with variations of pH in natural oral circumstances.

Conclusion

With the limitations of the study, Biodentine demonstrated superior bioactivity than Dycal and Lime lite in terms of calcium ion release, and there was no significant variation in pH after 48 hours between the three materials.

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