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Research Protocol

Dentistry Section

Comparative Evaluation of the Efficacy of Bromelain with Biodentine and Biodentine as Isolation Materials for Direct Pulp Capping: A Research Protocol

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ABSTRACT

Introduction: Direct Pulp Capping (DPC) aims to retain the pulp's health by sealing it against microbes and stimulating the formation of a dentin bridge at the exposure site. It has been proven that natural antimicrobial proteins, when used as therapeutics in conjunction with traditional chemotherapeutic agents, have increased efficacy and presumably diminished the incidence of chemoresistance. Hence, the efficacy of Bromelain with Biodentine and Biodentine as isolation materials for DPC will be evaluated and compared in the present study. The success and durability of DPC depend on the type of material used. Thus, the present study focuses on evaluating and considering a combination of materials for DPC that can be used in clinical practice.

Need for the study: By addressing the limitations of current materials and formulating a novel combination, the present study aims to contribute to the advancement of material science, providing solutions that meet the demands of this era. Such a breakthrough would not only enhance the performance and longevity of materials but also have a significant positive impact on the preservation of pulp vitality, promoting overall well-being and improved outcomes in healthcare applications.

Aim: To evaluate and compare the efficacy of Bromelain with Biodentine and Biodentine in isolation as DPC materials.

Objectives: The study aims to evaluate and compare the setting time, flow, solubility, compressive strength, adhesion, and radiopacity of bromelain with biodentine and biodentine in isolation.

Materials and Methods: An in-vitro study will be conducted in the Department of Conservative Dentistry and Endodontics at Sharad Pawar Dental College and Hospital, Wardha, Maharashtra, India, for a duration of nine months from May 2023 to February 2024. Standard discs of freshly mixed materials (n=10 samples per material) will be prepared. Compressive strength, solubility in distilled water, setting time, flow, radiopacity, and adhesion of Bromelain with Biodentine and Biodentine in isolation will be evaluated and compared in accordance with the International Standard ISO 6876:2001. Results will be analysed using Tukey's Honest Significant Difference (HSD) posthoc test, one-way Analysis of Variance (ANOVA), and student's t-test.

Keywords: Adhesion, Compressive strength, Flow, In-vitro, Radiopacity, Setting time, Solubility

INTRODUCTION

Direct Pulp Capping (DPC) is a method in which the exposed pulp is capped with a biocompatible material applied directly over the exposure spot. The primary goal of this treatment is to maintain the pulp's vitality by preventing bacterial invasion and promoting the development of a calcific barrier simultaneously [1]. Capping the breached odontoblastic layer induces reparative dentin production mediated by odontoblast-like cells (DPSCs) at the Materio-pulpal Complex (MPC) and acts as a "biological seal" to protect the underlying pulp tissues [2]. The use of DPC for mature permanent teeth with carious pulpal exposure is a subject of debate [1]. To enhance healing, repair, and protect the pulp from further damage, a coronal seal is essential. Several biocompatible materials have been developed as pulp-capping agents, including gold foil, calcium hydroxide, zinc oxide eugenol cement, bonding agent, tricalcium phosphate, dentin shavings, growth factors, mineral trioxide aggregate, theracal, and biodentine [3].

Biodentine™, developed by Septodont in Saint Maur des Fosse's, France, is a recently introduced dentin substitute. It consists of a powder enclosed in a capsule and a liquid contained in a pipette. The powder primarily contains tricalcium and dicalcium silicate, and calcium carbonate. Zirconium dioxide is added as a contrast medium. The liquid is composed of an aqueous solution of calcium chloride with an admixture of polycarboxylate. The powder and liquid are mixed together in a capsule using a triturator for 30 seconds. As the cement sets, calcium hydroxide is produced. Biodentine can be used for various dental procedures, such as pulp capping, sealing perforations and resorption, root-end fillings, and pulpotomy [4]. Enzymes like hemeoxygenase have also been employed for DPC [3]. One bioactive enzyme is Bromelain, which is a proteolytic enzyme extracted from the stems of pineapple plants [5]. Bromelain has a strong therapeutic potency with a variety of proteinase inhibitors [6]. It acts as a predominant oxidising agent [7]. Its well-established effects include antithrombotic, fibrinolytic, antifungal, proteolytic, antibacterial, and anti-inflammatory properties [8]. It also exhibits anticancer functions [6].

Bromelain has been extensively used not only in the medical field but also in the dental industry. Dental applications of bromelain include pain, erythema, and inflammation reduction after third molar extraction [9], intracanal medicament [8], dentin deproteinisation [10], and enamel bleaching [7]. Additionally, bromelain has been documented as a synergistic agent with traditional medicaments such as ampicloxacillin, dexamethasone sodium phosphate, and acetylcysteine, enhancing the efficacy of these conventional agents [11]. However, no study has yet compared the efficacy of Bromelain with Biodentine and Biodentine in isolation as DPC materials. Several materials are available for DPC, and evaluating their effectiveness involves assessing physical, chemical, and biological properties. Key properties include setting time, flow, solubility, adhesion, radiopacity, and compressive strength [4].

In DPC, a shorter setting time is crucial to achieve a tight seal between dentin and the capping material. Prolonged setting times can lead to inconsistency in the mixture [4]. Solubility is important for dental restorative materials. Low solubility is desirable for long-term durability and sealing. However, DPC agents should exhibit some solubility to promote mineralisation near vital tissue while maintaining an effective seal [4]. Compressive strength is essential as these materials endure condensation pressure and masticatory loads. Adequate strength ensures durability during placement and function [12]. Radiopacity is necessary for quality assessment, with International Organisation for Standardisation (ISO) standards requiring a minimum radiopacity of 3 mm aluminum to differentiate materials from dentin [13]. Flow and adhesion are crucial for material selection. Good flowability allows penetration into dentinal tubules, ensuring adherence to dentin. This supports proper sealing, dentin bridge formation, prevents microleakage, and minimises contamination, all of which are essential for successful vital pulp therapy [14].

The success and durability of DPC depend on the type of material used. Thus, the present study focuses on evaluating and considering a combination of materials for DPC that can be used in clinical practice. Therefore, the aim of the study is to evaluate and compare the efficacy of Bromelain with Biodentine and Biodentine in isolation as DPC materials.

Primary Objectives

- To evaluate the setting time of Bromelain with Biodentine and Biodentine in isolation.
- To evaluate and compare the solubility of Bromelain with Biodentine and Biodentine in isolation.
- To assess and compare the compressive strength of Bromelain with Biodentine and Biodentine in isolation.
- To assess and compare the adhesion of Bromelain with Biodentine and Biodentine in isolation.

Secondary Objectives

- To evaluate and compare the flow of Bromelain with Biodentine and Biodentine in isolation.
- To assess and compare the radiopacity of Bromelain with Biodentine and Biodentine in isolation.

Null hypothesis: The setting time, flow, solubility, compressive strength, adhesion, and radiopacity of the combination of bromelain with biodentine will not show any significant difference when compared with biodentine in isolation.

Alternative hypothesis: There will be a significant difference in setting time, flow, solubility, compressive strength, adhesion, and radiopacity between the combination of bromelain with biodentine and biodentine alone.

REVIEW OF LITERATURE

During the treatment of deep caries, preserving the pulp's vitality is crucial [15]. The healthy pulp has the inherent ability to heal and recover from a damage, making it essential for the long-term health of a tooth [16]. Various materials have been used in the past for capping exposed pulp and performing pulpotomy treatments [17]. An ideal pulp-capping material should retain pulp vitality and promote the production of reparative dentin [16]. It should also be biocompatible, bioactive, have antibacterial properties, and minimise microleakage to aid in the repair of the injured tooth pulp [18,19]. Although various direct pulp-capping materials have been developed to simulate biological healing, many of them lack all the desired characteristics [20].

One such material is Biodentine, a resin-free restorative cement based on Tricalcium Silicate (Ca_3SiO_5) [21]. Biodentine offers several advantages, including the preservation of pulp vitality, ease of handling, short setting time, and dentin remineralisation [22]. It promotes pulp repair and early mineralisation by releasing Transforming Growth Factor 1 (TGF-1) and stimulating odontoblasts. Furthermore, the silicon ions generated by Biodentine play a crucial role in the mineralisation of the dentinal bridge [23]. Biodentine promotes the formation of a dentin bridge without causing any inflammatory pulpal reactions. This is attributed to its anti-inflammatory effect, which inhibits the release of proinflammatory mediators and reduces the recruitment of inflammatory cells [21]. Due to its suitable setting time and restorative properties, Biodentine has shown significant potential as a pulp-capping material [24]. In a recent study by Katge FA and Patil DP, Biodentine demonstrated 100% effectiveness in carious young permanent molars after one year of follow-up [25].

Butt N et al., evaluated the initial setting time and compressive strength of Biodentine. They found the setting time to be 6.5 minutes and the compressive strength to be 139.5 MPa, 170.78 MPa, 269.08 MPa, and 304.78 MPa at one hour, one day, seven days, and 28 days, respectively [4]. In a laboratory study conducted by Kaup M et al., aimed at evaluating the material properties of Biodentine, it was found to be less soluble than 3% after 24 hours. The radiopacity of Biodentine was measured at 1.50 mm of aluminum thickness, and the final setting time was 85.66 minutes [26].

Bromelain is a proteolytic enzyme (protease) that breaks proteins down into amino acids [8]. It is widely available and used as a phytomedicine [27]. Bromelain contains various proteinase inhibitors, giving it significant therapeutic potential [6]. It is also an oxidising agent [7]. Bromelain has been shown to possess antifungal, fibrinolytic, antibacterial, antithrombotic, anti-inflammatory, and even anticancer properties [8].

The synergistic effects of bromelain have been studied not only in medicine but also in dentistry. Ordesi P et al., concluded from their study that bromelain reduces pain, erythema, and inflammation after third molar extraction [9]. Chauhan K et al., found that bromelain causes dentin deproteinisation before the application of the adhesive system, thereby increasing bond strength [10]. A study conducted by Chandwani ND et al., showed that bromelain exhibits antibacterial action against Enterococcus faecalis, a prominent bacterium causing root canal infections [8]. Vekaash CJV et al., concluded from their study that bromelain, when used in combination with hydrogen peroxide, enhances the bleaching of human enamel [7]. Due to its anti-inflammatory and antibacterial properties, bromelain has the potential to be a promising additive to Biodentine, increasing its efficacy and the longevity of the treated tooth.

MATERIALS AND METHODS

The present in-vitro study will be conducted in the Department of Conservative Dentistry and Endodontics at Sharad Pawar Dental College and Hospital in Wardha, Maharashtra, India. The study will have a duration of nine months, from May 2023 to February 2024. The research methodology has been approved by the Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences (Deemed to be University) in Sawangi (Meghe), Wardha, Maharashtra, India, under the reference number DMIMS(DU)/IEC/2022/768, dated February 2022.

Inclusion criteria: Premolars extracted for orthodontic reasons will be included in the study. Teeth without any restorations, cracks, or fractures will be considered.

Exclusion criteria: Teeth with caries, restorations, cracks, or fractures will be excluded from the study.

Sample size calculation: The sample size was calculated using Open Epi software (version 3.04.04) based on a study conducted by Kaup M et al., at a 95% confidence interval and 80% power, using the mean solubility of Biodentine in distilled water [4]. The calculated sample size is 26.

Formula:

$$N=\frac{(\sigma_1^2+\sigma_2^2/\kappa)\ (Z_{1-\alpha/2}+Z_{1-\beta})^2}{\Delta^2}$$

Notation for the formula:

N=sample size

σ_1=Group 1 standard deviation (0.2) [4]

σ_2=Group 2 standard deviation (0.42) [4]

Δ=mean differences across groups

κ=ratio=1

$Z_{1-\alpha/2}$=two-sided Z value (e.g., Z=1.96 for a 95% confidence interval)

Z_1-β=power

Using the formula, the sample size is calculated as follows:

$N=(0.2\times0.2)+(0.42\times0.42)\ (1.96+0.84)^2/0.44\times0.44=9$

The sample size per group is found to be 9 [4].

Thus, a total sample size of 120 will be required, with 10 samples in each of the 12 groups [Table/Fig-1].

Groups	Sample
Group-1A	10 samples to evaluate the setting time of Bromelain with Biodentine.
Group-1B	10 samples to evaluate the setting time of Biodentine.
Group-2A	10 samples to evaluate the solubility of Bromelain with Biodentine.
Group-2B	10 samples to evaluate the solubility of Biodentine.
Group-3A	10 samples to evaluate the compressive strength of Bromelain with Biodentine.
Group-3B	10 samples to evaluate the compressive strength of Biodentine.
Group-4A	10 samples to evaluate the adhesion of Bromelain with Biodentine.
Group-4B	10 samples to evaluate the adhesion of Biodentine.
Group-5A	10 samples to evaluate the flow of Bromelain with Biodentine.
Group-5B	10 samples to evaluate the flow of Biodentine.
Group-6A	10 samples to evaluate the radiopacity of Bromelain with Biodentine.
Group-6B	10 samples to evaluate the radiopacity of Biodentine.

[Table/Fig-1]: Sample distribution.

In the present study, a total of 120 samples will be divided into 12 groups as follows:

Primary outcomes: The primary outcomes will include setting time, solubility, compressive strength, and adhesion.

Secondary outcomes: The secondary outcomes will include flow and radiopacity.

Procedure: A combination of bromelain (Brisk Bioscience, Surat, Gujarat, India) and Biodentine (Septodont, Saint-Maur-des-Fossés, France) powders will be mixed in a 1:1 ratio.

To evaluate and compare the setting time of Bromelain with Biodentine and Biodentine in isolation:

a. The procedure outlined in ISO 9917-1:2007 will be used to evaluate the setting time.

b. The cement will be mixed and compacted into stainless steel rectangular molds with a depth of 5mm and a cross-section of 10 mm by 8 mm.

c. The specimens will be kept at 37°C.

d. To assess the setting time, a modified Vicat apparatus will be utilised, consisting of a weighted needle with a square cross-section of size 1±0.01 mm and a total mass of 400±5 g.

e. The cement will be inspected for setting at 15-minute intervals initially.

f. The ultimate setting time will be determined as the period from the start of mixing to the time when the indenter fails to leave a mark on the set cement surface.

To evaluate and compare the Solubility of Bromelain with Biodentine and Biodentine in isolation:

a. Specimens with a thickness of 1±0.1 mm and a diameter of 15±1 mm, as specified in ISO 4049:2009, will be prepared.

b. The materials will be manipulated, poured into the moulds, and allowed to set for 24 hours at 37 degrees Celsius.

c. The specimens will then be demoulded and weighed with an accuracy of ±0.1 g to record their mass 'm'.

d. The mean diameter and thickness of each specimen will be measured with a precision of 0.01 mm, and the volume 'V' of each specimen will be determined.

e. The specimens will be submerged upright in 10 mL of Hank's Balanced Salt Solution.

f. After one day, the specimens will be removed and dried using filter paper. One minute after removal from the storage solution, the specimens will be weighed with an accuracy of 0.1 g. Their mass will be assigned as 'm1'.

g. After 7 and 28 days, the same technique will be repeated to determine the solubility of the specimens, and their masses will be recorded as 'm2' and 'm3'.

h. Fluid solubility (Fsl) will be evaluated for each sample using the equation Fsl (%)=(m-m1/2/3/V)×100.

To assess and compare the compressive strength of Bromelain with Biodentine and Biodentine in isolation:

a. Compressive strength will be determined as suggested by ISO 9917-1:2007.

b. Cylindrical specimens measuring 4±0.1mm in diameter and 6±0.1 mm in height will be made and submerged for 28 days in gelatinised Hank's Balanced Salt Solution at 37°C.

c. After that, they will be evaluated in a universal testing machine (Lloyd LR MK1 machine; Lloyd Instruments, Fareham, UK), first within the initial hour and then again after 28 days.

To assess and compare the adhesion of Bromelain with Biodentine and Biodentine in isolation:

According to the study performed by Atmeh AR et al., the adhesion of both samples to dentin will be assessed in the following manner [14]:

a. Five mandibular premolar teeth extracted for orthodontic purposes will be taken, and 10 dentin discs of thickness 1 mm each will be made.

b. Biodentine will be applied to five of the resulting discs, while a combination of bromelain and biodentine will be applied to the others.

c. These discs will then be placed in distilled water at a temperature of 37°C for a duration of four days.

d. Afterwards, the discs will be fractured perpendicular to the interface and coated with a thin layer of gold using a process called gold-sputter-coating.

e. The fractured surfaces of the discs will be examined using scanning electron microscopy (Hitachi S3500N model from Hitachi High Technologies in Maidenhead, UK).

To evaluate and compare the flow of Bromelain with Biodentine and Biodentine in isolation:

a. The flow test will be carried out in compliance with ISO 6876:2002.

b. Using a graduated syringe, 0.05 mL of cement will be placed in the center of a glass plate after manipulating the material.

c. Another glass plate (20 grams) will be placed on the previous plate with cement at 180±5 seconds after starting the manipulation, and a 100 g weight will be placed on the top plate and held in the same position for 10 minutes.

d. As time elapses, the material's greatest and lowest diameters over the plate will be measured.

e. The mean value will be recorded when the difference between the diameters is smaller than 1 mm.

f. The material on the plate will be photographed next to a millimeter ruler for a second inspection.

g. The photos will be analysed to determine the material's flow area and will be represented in mm^2.

h. Three repetitions will be done for each group.

To assess and compare the radiopacity of Bromelain with Biodentine and Biodentine in isolation:

a. In accordance with ISO 6876:2001, stainless steel ring moulds with a height of 1.0 mm (±0.1 mm) and an interior diameter of 10.0 mm (±0.1 mm) will be used for sample preparation.

b. Cement samples will be prepared and allowed to cure for 24 hours before being placed on a dental X-ray film with an aluminum step wedge (1-9 mm).

c. X-ray exposures will be made, and an automatic processor will be used to process the film.

d. The densities will be measured with a densitometer (DEN-1, McFarland Densitometer, Latvia, Europe).

STATISTICAL ANALYSIS

Open Epi software (Version 3.04.04) will be used for data analysis. The data for the primary outcomes will be tested for normality using the Kolmogorov-Smirnov test. Outcomes such as setting time, flow, radiopacity, adhesion, solubility, and compressive strength will be analysed using Tukey's HSD posthoc test, Student's t-test, and one-way ANOVA test to determine statistical significance at a p-value ≤ 0.05. If the primary variable (parameters) does not follow a normal distribution, it will be transformed using mathematical algorithms or functions such as log, exponential, square root, or box cox to achieve normality. If the data still does not exhibit a normal distribution, alternative non parametric tests such as the Mann-Whitney and Kruskal-Wallis tests will be used.

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