Research Protocol

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Dentistry Section

Comparative Evaluation of Novel Calcium Hydroxide-hydrogel with Calcium Hydroxide-iodoform as Obturating Material: A Protocol for an In-vitro Study

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ABSTRACT

Introduction: Pulpectomy is one of the extensively done procedures in clinical practice, in which the pulp is extirpated, and the canal is filled with an obturating material. Modalities of pulp therapy in primary teeth are subtly different from that of permanent teeth, and so are the obturating materials. Calcium hydroxide has an optimum antimicrobial effect on the root canal of teeth. The success rate for $Ca(OH)_2$-iodoform paste ranges from 84% to 100%. A mixture of calcium hydroxide with iodoform is conventionally used due to its strong antibacterial property, but, high resorptive activity leads to increased periapical radiolucency in the long term. Chitosan, a hydrogel, is antimicrobial, antioxidant and anti-inflammatory.

Need of the study: Calcium hydroxide-hydrogel can be potential alternative to the available calcium hydroxide iodoform obturating material for primary teeth by increasing its longetivity in the canal and giving a uniform ion release.

Aim: To prepare, optimise and characterise novel calcium hydroxide-hydrogel as an obturating material and compare it with conventional calcium hydroxide-iodoform.

Materials and Methods: The present in-vitro study will be carried out at Sharad Pawar Dental College, Wardha, Maharashtra, India. Chitosan-calcium hydroxide hydrogel will be formulated by soaking commercially available chitosan in 2% acetic acid solution. Calcium hydroxide will be added to this base in different concentrations for each sample and mixed in a magnetic stirrer. Twenty samples of calcium hydroxide-hydrogel will be formulated and divided into three groups with concentration of 0.1, 0.55 and 1% w/v, respectively and a control group with calcium hydroxide-iodoform will also be evaluated. Calcium ion release shall be checked through infrared spectroscopy techniques and pH through pH meter at 7, 14, 21, and 28 days. To evaluate, an Analysis of Variance (ANOVA) followed by post- a hog test will be used.

Keywords: Chitosan, Hydrogel, Metapex, Pulpectomy

INTRODUCTION

Primary teeth have a wide range of functions like mastication, conservation of arch integrity, facilitation of jaw growth, speech, and aesthetics. Caries incidence is high in children, which may involve pulp due to ignorance. Therefore, pulpectomy being one of the extensively done procedures in clinical practice, demands a significant concern. It is indicated in cases of necrotic pulp and where the infection has reached the pulp canal of primary teeth. After complete extirpation of the pulp, the canal is vigorously cleaned with an irrigating solution and packed with an obturating material [1,2]. An obturating material, should have antimicrobial properties, be radiopaque, and be inert to developing tooth buds. Also, in the case of the primary tooth, it should have the same resorptive rate as that of the roots of teeth. Apart from these, the material should adhere to the canal walls, and in case it passes beyond the root apex, it should readily absorb without producing any inflammatory response. Pulpectomy's success is ensured by filling the obturating material throughout the proper length of the canal, absence of voids, and establishing an excellent apical seal. Various materials for obturation like zinc oxide eugenol, calcium hydroxide formulations, iodoform-based preparations, etc., have been introduced [3-5].

Due to potent pharmacological properties and biological activity, calcium hydroxide has an optimum antimicrobial effect on the root canal of teeth, but it is challenging to use it solely for obturation because of its poor handling characteristics. Conventionally, metapex, a mixture of calcium hydroxide with iodoform and silicon oil is used due to its substantial antibacterial property and prolonged leaching of calcium ions. But the high resorptive activity of metapex leads to increased periapical radiolucency in primary teeth in the long term [6,7].

Chitosan, the end product of the deacetylation of chitin, is a hydrogel. Crustacean's exoskeleton is a significant source of chitin. To date, chitosan has been used as an adhesive agent for restorative purposes, denture base adhesive, irrigant, localised drug carrier, etc. It can be used as a vehicle for modifying the handling properties of calcium hydroxide in the form of an injectable gel [8]. Chitosan is poorly soluble in water rendering it fit for usage as an obturating material in the root canal. Apart from this, it has been shown to have antimicrobial, antioxidant, anti-inflammatory, and healing properties [7]. These properties add to the effects of calcium hydroxide. Because of the chemical structure and active chemical groups, chitosan can be modified to satisfy specific needs based on the intended usage, such as adequate endurance, mechanical qualities, or biodegradability time [9].

Chitosan, as a vehicle, has been shown to release of calcium ions from calcium hydroxide in a controlled manner for prolonged time. These characteristics make it a good vehicle for calcium hydroxide. Chitosan being synergistic with calcium hydroxide has proven to be a potent antimicrobial [4]. Calcium hydroxide-iodoform in long-term assessment shows a faster depletion of calcium from the roots of primary teeth [3,10].

The aim of the study is to prepare, optimise and characterise novel calcium hydroxide-hydrogel as an obturating material and compare it with conventional calcium hydroxide-iodoform.

The objectives therefore, would be to optimise the formulation of novel calcium hydroxide-hydrogel to be used as obturating material,

to check the pH and release profile of calcium ions in novel calcium hydroxide-hydrogel and compare it with calcium hydroxide-iodoform.

REVIEW OF LITERATURE

The significance of $Ca(OH)_2$ in the endodontic treatment of teeth is well acknowledged. It has been used in various combinations like Endoflas, Metapex, Vitapex, etc., [10]. To further improvise the longevity of calcium hydroxide, this study plans to combine it with chitosan, a hydrogel that shall be dissolved in acetic acid. Grover C et al., evaluated the environmental pH change and the calcium ion release when calcium hydroxide was incorporated in vehicles like propylene glycol, distilled water, chitosan, and gutta purcha. For the above-mentioned study, 40 mandibular premolar teeth were taken, and the canals were obturated with calcium hydroxide mixed with distilled water, propylene glycol, gutta purcha points, and chitosan-based on groups. Calcium ion release and pH were checked, wherein at the end of 15 days, group one showed almost complete ion release, group three most minor, whereas group four showed sustained release of Ca ions [10].

Shaikh J et al., also conducted an in-vitro study to comparatively evaluate the antimicrobial efficacy of triple antibiotic paste (TAP) with calcium hydroxide using chitosan as a carrier against C.albicans and E.faecalis, for which 80 single-rooted anterior teeth were selected. They were segregated into four groups: TAP+Saline (group 1), TAP+chitosan (group 2), Calcium hydroxide+chitosan (group 3), Calcium hydroxide+saline (group 4). The study concluded that combinations of TAP and Calcium hydroxide with chitosan produced better results when compared with that saline [7].

A study was conducted by Farhadian N et al., to in-vitro prepare and characterise chitosan/gelatin nanocarriers for calcium hydroxide to modify its therapeutic effects utilised chitosan powder dissolved in 1% acetic acid and pH was adjusted to 5 by NaOH addition. Drug loading, particle size, polydispersity index, and encapsulation efficiency were investigated for the samples. The results showed that the particle size of different polymeric nanocarriers increased by increasing the calcium hydroxide concentration, whereas the viscosity was proportional to the amount of chitosan in the solution. Sustained release of calcium hydroxide was observed during the experimental period that at the end of 300 hours was 72%. Therefore, it was concluded that the calcium hydroxide-loaded polymeric nanocarriers are suitable for root canal therapy with the advantage of prolonged release [6]. Flores-Arriaga JC et al., performed a study to formulate chitosan paste loaded with calcium chloride or calcium hydroxide and evaluate the calcium ion release, pH changes, and cell viability. The pastes were prepared by adding calcium chloride or calcium hydroxide into chitosan dissolved in 1% or 2% acetic acid. Calcium hydroxide with polyethylene glycol was taken as the control. Dialysis tubing was used to evaluate Ca^{2+} release and a pH meter for pH testing. In the tests, the highest release of ions was observed in control and the lowest in chitosan- calcium chloride dissolved in 2% acetic acid. The most elevated pH was seen with calcium chloride mixed with chitosan dissolved in 2% acetic acid. It was seen that the pH decreased with time. The cell viability test proved that none of the pastes caused cell damage. The study concluded that the chitosan-based pastes were cyto-compatible and gave a sustained release of ions [11].

Nunes B de S et al., conducted a study to develop chitosan-based biomaterial using calcium hydroxide and 2% Chlorhexidine (CHX) for intracanal medicament for the root canal system. For the study, they used six different concentrations of chitosan, calcium hydroxide, and 2% CHX, "M1: $Ca(OH)_2$+Q2%; M2: $Ca(OH)_2$+Q4%; M3: $Ca(OH)_2$+Q2%+CLX; M4: $Ca(OH)_2$+ Q4%+CLX; M5: $Ca(OH)_2$+ Q2%+PEG; and M6: $Ca(OH)_2$+Q4%+PEG," wherein 2g of chitosan was dissolved in 100 mL of 2% glacial acetic acid (Q2) and another being solution of 4g chitosan in 100mL of 4% glacial acetic acid (Q4). The formulations were analysed through rheological measurement, X-ray diffraction, and Fourier transforms infrared spectroscopy. Along with these, the antimicrobial activity was evaluated in-vitro. The X-ray diffraction technique showed that the material has a semi-crystalline structure. The viscosity test confirmed the pseudoplastic behaviour of the material, whereas the result of the microbial analysis was positive for all samples. Still, because of the synergistic impact of chitosan, calcium hydroxide, and CHX, greater antibacterial activities were noted for the M3 and M4 samples. According to the results, it was concluded that chitosan formulations have the potential to be used as an intracanal medication [12]. For the study, commercially available low molecular weight chitosan powder will be procured and mixed with 1% acetic acid so as to convert it into gel form. The study will help characterise calcium hydroxide with hydrogel as a delivery agent by finding its proper concentration at which the calcium ions release is maximum along with optimum pH.

MATERIALS AND METHODS

This in-vitro study will be carried out at Sharad Pawar Dental College, Wardha, Maharashtra, India. Ethical approval for the study was obtained from the Institutional Ethics Committee of Datta Meghe Institute of Medical Sciences (ref. no: DMIMS(DU)/IEC/2022/757).

Sample size calculation: The sample size calculation is based on previous research by Ballal NV et al., using a similar technique and using the sample size formula for absolute difference between two means [8]. Twenty samples of calcium hydroxide formulations will be included in the study, randomly divided into five groups (experimental group with four subgroups and one control group). The sample size is for the different number of formulations that will be made of calcium hydroxide-chitosan, which shall constitute the experimental group and the control group i.e., Calcium hydroxide-iodoform.

Study outcome: Calcium hydroxide-hydrogel will prove to be more efficient than calcium hydroxide-iodoform as obturating material and will substitute the conventional calcium hydroxide-iodoform mixture.

Study Procedure

The method for sample preparation for calcium ion release and pH measurement as described by Flores-Arriaga JC et al., will be followed [6,8,11]. Commercially, available chitosan will be soaked in (2% v/v) acetic acid. Calcium hydroxide will be added and mixed in a magnetic stirrer to this base. Barium sulfate shall be used as an opacifier to make the material radioopaque. The mixture will be then filled in a microcapillary tube to mimic root canals. For the control group, commercially available Calcium hydroxide- iodoform i.e., Metapex shall be used. The tubes shall then be kept in a glass vial filled with distilled water.

The samples of the experimental group will then be divided into four sub-groups, with four samples in each group based on the concentration of calcium hydroxide and chitosan, and one group shall consist of calcium hydroxide-iodoform. In group 1, 1g Calcium hydroxide will be mixed with 5, 7, 9 and 11g of chitosan, respectively. Similarly, for group 2, 3g Calcium hydroxide; group 3, 5g and group 4, 7g Calcium hydroxide will be mixed with 5, 7, 9, 11g of chitosan. They will then be characterised through infrared spectroscopy technique. An ultraviolet spectrophotometer shall be used to check for the concentration of calcium ion release from distilled water in which the microcapillary tubes shall be placed, whereas pH shall be checked through a pH meter. The samples shall be examined at 7, 14, 21, and 28 days for calcium ion release and pH [6,11].

STATISTICAL ANALYSIS

The data will be analysed with Statistical Package for Social Sciences (SPSS) version 22.0 (IBM SPSS, Chicago, IL, USA). To evaluate the effect of calcium ion release and pH in each group, an ANOVA followed by post-a hog test will be used.

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