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Evaluation of Properties of Retrograde Filling Materials

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Abstract

Aim: The aim of the study is to evaluate the solubility, water sorption and bioactivity of conventional and modified retrograde filling materials after immersion in simulated body fluid (SBF).

Methodology: Silver, Chitosan, calcium hydroxide and hydroxyapatite nanoparticles synthesized. The nanoparticles were subjected to XRD analysis and incorporated into the powder of conventional glass ionomer cement and mixed according to the manufacturer's instructions and solubility, water sorption and bioactivity of the materials were assessed.

Results: The results were tabulated and subjected to statistical analysis. Kruskall wallis test is performed based on variables among all 6 groups. The Alternative hypothesis would be there is significant difference between 6 groups and the opposite would be null hypothesis. P value less than 0.05 shows that all groups are significantly different from each other based on solubility, calcium release and water sorption.

Conclusion: Chitosan had the highest calcium release and biodentin had the lowest calcium release after 24 hours. Hydroxyapatite had the highest calcium release and biodentin had the lowest calcium release after 7 days. Chitosan had the highest solubility and pro root MTA had the lowest solubility after 24 hours. Chitosan had the highest solubility and pro root MTA had the lowest solubility after 7 days. Pro root MTA had the highest water sorption and Calcium hydroxide had the lowest water sorption after 24hours. Pro root MTA had the highest water sorption and Calcium hydroxide had the lowest water sorption after 7days.

Keywords: bioactivity, nanoparticles, solubility, retrograde filling materials, water sorption

Introduction

When the well-known complexity of the root canal system and the acknowledged difficulty of completely eliminating all potential irritants from the canal system are considered, it is remarkable that conventional nonsurgical root canal therapy enjoys a predictably good rate of clinical success (1). According to Sjögren.U et.al and Swartz DB et.al, the clinical success after nonsurgical root canal treatment ranges from 86% to 96%. Clinical success can be defined as follows: absence of symptoms; absence of swelling, sinus tract, and other signs of infection; radiographic evidence of healing; and continued normal functioning of the tooth (1)

Although endodontic treatment is a predictable procedure with high success rates, failures can occur either through persistent infection or through recontamination of the root canal system (7). Identifying the cause of the failure is the first step and the key to establishing an appropriate treatment plan. Management is directed at eliminating the source of the failure, which is most commonly the presence of bacteria and other irritants in an incompletely cleaned and/or poorly obturated canal. Peri-radicular surgery is indicated when nonsurgical re-treatment is impractical or unlikely to improve on the previous result. (1) Surgical endodontic therapy involves the exposure of the involved area, preparation of the root end cavity and placement of root end filling material to seal the canal (2).

Recently, several new root-end filling materials have emerged to challenge the pre-eminence of amalgam as the root-end filling material of choice (1). In the past, many materials such as amalgam, zinc phosphate cement, gold foil were used as retrograde filling material. But because of their limitations like marginal leakage, lack of corrosion resistance and irritation to periapical tissues, they have been replaced by newer materials.

Gray and tooth-colored (also known as white) ProRoot MTA (Dentsply Tulsa Specialties, Tulsa, OK) has revolutionized root-end therapy and pulp capping procedures. The advantages include its ability to set in moist/bloody environments or sites contaminated by biological fluids, its ability to stimulate the formation of hydroxyapatite and the generation of a flux of calcium and hydroxyl ions through dentin. (5,6) On the other hand, MTA has several disadvantages such as; long setting time, difficult handling, expensive, potential discoloration, and lower compressive and flexural strengths (Unal et al., 2010; Negm et al., 2017). A study performed by Torabinejad M et al., did not reveal any significant solubility of MTA whereas, Fridland M and Rosado R have reported the significant increase in solubility and porosity of ProRoot MTA with the increase in water to powder ratio (36, 37).

Biodentine™ was developed by Septodont's Research Group, which had high mechanical properties with excellent biocompatibility, as well as a bioactive behavior. Biodentine™ turns out to be one of the most biocompatible of all the biomaterials in dentistry as demonstrated according to all the ISO standard tests, as well as in the different preclinical and clinical research collaborations. Moreover, reactionary dentine formation was demonstrated in rats, exhibiting high quality and quantity of protective dentine stimulation in indirect pulp capping (4) Biodentine has a number of advantages over MTA, a few being faster setting time, lesser porosity, better compressive and compressive strength but had less radio-opacity when compared to MTA.

Glass ionomer is a hybrid of the silicate and polycarboxylate cements, which bond physicochemically to dentin and enamel, and possess anti cariogenic activity.

Some of its advantages are good biocompatibility, it has tight sealing ability (Chong et al 1995), dentin bonding is through chemical adhesion and it has easy handling. Sometimes it causes insufficient filling and hollow spaces form between cavity wall and filling (Khoury & Staehle 1987). It is highly sensitive to moisture and drying during the first handling stage (3).

GICs were initially produced as conventional GIC and then the addition of metals to the filler component in order to reinforced GIC (R-GIC) had been proposed. As a result, it had been more radiopaque. The negativity of conventional GIC is sensitivity of moisture especially when it hardens and it lacks toughness. This limitation has been addressed through the introduction of hybrid GICs as the resin-modified GIC and polyacid-modified composite resins (PM-CR). A few advantages include, they do not induce inflammatory tissue responses; they also present good sealing properties because of their ability to form a chemical bond with dentine. These cements generate no heat while setting; they will not cause thermal damage to tissues and will not affect heat-labile drugs incorporated in the matrix phase of the cement (38) Some studies have reported incomplete sealing with GIC, suggesting that it may be sensitive to contamination by saliva and blood, thereby suffering some disintegration (39,40). Some authors found GIC to have a higher leakage index when not photopolymerized, which could be a result of being exposed to oral fluids in its most vulnerable state (41).

For a long time silver has been known to have a disinfecting effect and its salts and their derivatives are commercially employed as antimicrobial agents (7). Gomes-Filho et al. evaluated the tissue response to implanted polyethylene tubes filled with fibrin sponge embedded with nanosilver dispersion. They concluded that nanosilver dispersion was biocompatible, mainly at low concentrations. Therefore, low concentration (1% by weight) and small particles (<150nm) were used in this study to reduce toxicity (8). The results of Samiei et al. study revealed that adding nanosilver by 1% weight to MTA improve its antimicrobial activity against E. faecalis, C. albicans and P. aeruginosa.

In recent years, chitosan nanoparticles have emerged as potential carriers for delivery of drugs ranging from small organic molecules to proteins and nucleic acids. The chitosan nanoparticles can be easily dispersed in distilled water or aqueous buffers of neutral pH and exhibit higher antibacterial efficacy in comparison to chitosan. It is a non-toxic, inexpensive, and highly biocompatible biopolymer that can be easily biodegraded through different hydrophilic enzymes, promoting positive biological effects such as bactericidal, anti-inflammatory, antioxidant, antitumor, and healing properties (14-17).

Hydroxyapatite (HA) is one of the bioceramic materials that forms the principal mineral component of bone and comprises 60% to 70% of the calcified skeleton. HAp has been widely used in repair of hard tissues, and common uses include bone repair, bone augmentation, as well as coating of implants or acting as fillers in bone or teeth. However, the low mechanical strength of normal HAp ceramics generally restricts its use to low load-bearing applications. It has been suggested that nano HAp may be an ideal biomaterial due to its good biocompatibility and bone integration ability (12, 13).

Calcium hydroxide (CH) is widely used as an intracanal medicament. In order to be effective, the hydroxyl ions (OH-) in CH should diffuse into the dentinal tubules and accessory canals where the bacteria are harbored. The release of these ions induces an alkalizing effect and destroys the cellular membranes and protein structures. CH also dissolves the remaining tissue debris. It has the ability to promote an osteogenic environment and prevent root resorption (23-26)

The quality of the apical sealing obtained by root-end filling materials has been assessed in different ways such as degrees of dye penetration, bacterial penetration eletromechanical ways and fluid filtration technique. Studies on dye penetration were considered an easy method to evaluate root-end filling materials. However, Scanning electron microscopy (SEM) has also been used to assess the adaptation and the sealing capacity of commonly used root-end filling materials (10) SEM has larger depth of field, higher resolution, and better magnification at the interface which has been also pointed by Punithia and Shashikala. The SEM uses electromagnets rather than lenses allowing the researcher to have more control over the degree of magnification, there-by providing strikingly clear images (43, 44)

Ideal materials for sealing root-end cavities should prevent leakage. They should have dimensional stability, should adhere to the walls of the cavity, should be resistant to resorption, and should be moisture resistant; they should also be nontoxic and biocompatible to promote healing (42). The ability to release calcium ions able to diffuse through dentin and inside the surrounding tissues is a key factor for successful endodontic and pulp capping therapies because of the action of calcium on the differentiation of mineralizing cells as dental pulp cells , cementoblasts , osteoblasts, periodontal fibroblasts , mesenchymal stem cells, and hard tissue mineralization (5). Thus, the aim of the study is to evaluate the solubility, water sorption, marginal adaptation and bioactivity of conventional and modified retrograde filling materials after immersion in simulated body fluid (SBF).

Materials and methodology

Synthesis of nanoparticles

Procedure for the preparation of Ag Nanoparticles

Silver nitrate (Sigma, Aldrich) was dissolved in purified water. Polyvinylpyrrolidone (PVP) (Sigma, Aldrich) was further dissolved in water and mixed with silver nitrate solution. The reaction mixture was kept under ice bath (15 degrees C). Sodium borohydride solution (Sigma, Aldrich) was further added drop by drop to the silver nitrate solution. Black precipitate was obtained. The precipitate was further washed with deionised water for several times and dried under vacuum for 2 hours at 80 degrees C. The nanoparticles thus obtained were confirmed using XRD analysis.

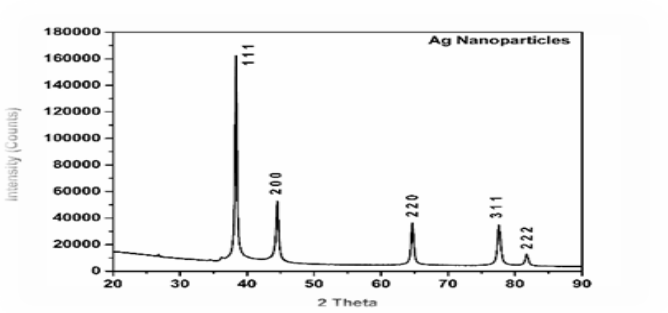

Figure 1: XRD image of Silver Nanoparticles

Procedure for the Preparation of Chitosan Nanoparticles

1% w/v Chitosan (Sigma, Aldrich) was dissolved in 5% acetic acid. To this 2% Thiamine pyrophosphate (TPP) Sodium solution (Sigma, Aldrich) was added drop wise under stirring for 4 hours at 500 rpm. The chitosan nanoparticles were washed and re-dispersed. The nanoparticles were then freeze dried. The nanoparticles thus obtained were confirmed using XRD analysis.

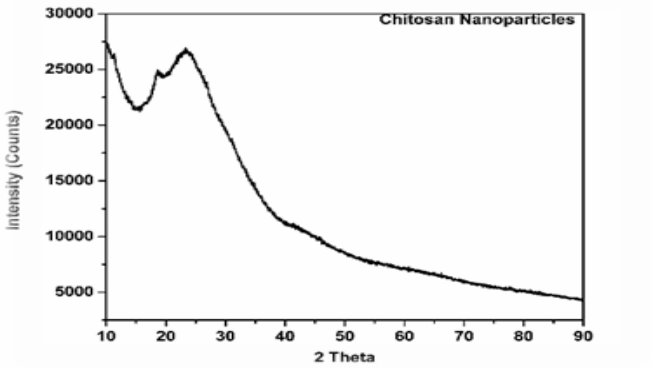

Figure 2: XRD image of Chitosan Nanoparticles

Procedure for the Preparation of Hydroxyapatite Nanoparticles

20 g of natural hydroxyapatite $3Ca_3(PO_4)2Ca(OH)_2$ powder was added in a solution consisting of 200 ml of ethanol and 50 ml of acetic acid. Then a solution of repeated units of 99% chemically pure grade polysaccharide (1–3 linked β-D galactopyranose and 1, 4 linked 3, 6 anhydro- α-L-galactopyranose) was used and added to this solution for gel formation. Later, the gel was placed in 1 mL tube and centrifuged at 12,000 rpm for 5 minutes at 28◦ C. Further centrifugal action to 12,000 rpm for 5 minutes was carried out to recover the powder precipitate, followed by a 48-hour period in an incubator at 28◦C to evaporate any residual water. After this, the powders were calcined in a laboratory muffle at 810◦ C for 2 hours. The nanoparticles thus obtained were confirmed using XRD analysis.

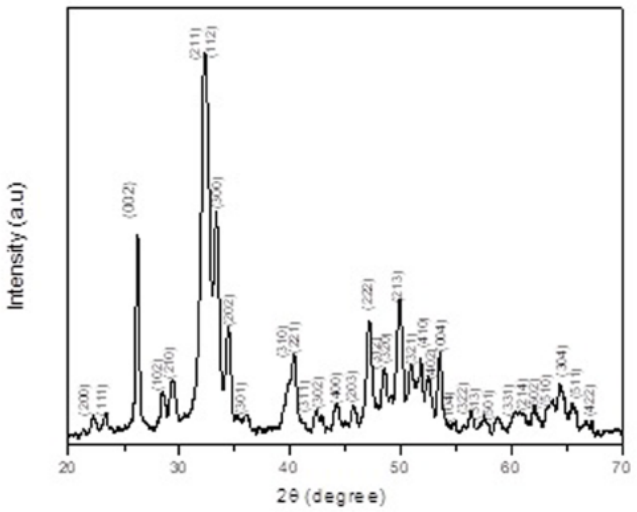

Figure 3: XRD image of Hydroxyapatite Nanoparticles

Procedure for the Preparation of Calcium hydroxide Nanoparticles

First, 250 mL of 0.4 M $Ca(NO_3)_2$ and 100 ml of Cyanuric fluoride (CNF) at various fiber and carboxylate concentrations were mixed and stirred for 10 min, and then 250 mL of 0.4 M NaOH was added drop by drop into the CNF suspension in $Ca(NO_3)_2$ solution. The color of the CNF suspension turned milky, and the mixture was stirred at 35 °C for a period of time. After the completion of reactions, samples were washed and centrifuged several times and redispersed in deionized water. Carbonation of $Ca(OH)_2$ nanoparticles on cellulose nanofibers was evaluated by transferring samples in Petri dishes, letting them dry under natural air convection. The nanoparticles thus obtained were confirmed using XRD analysis (18).

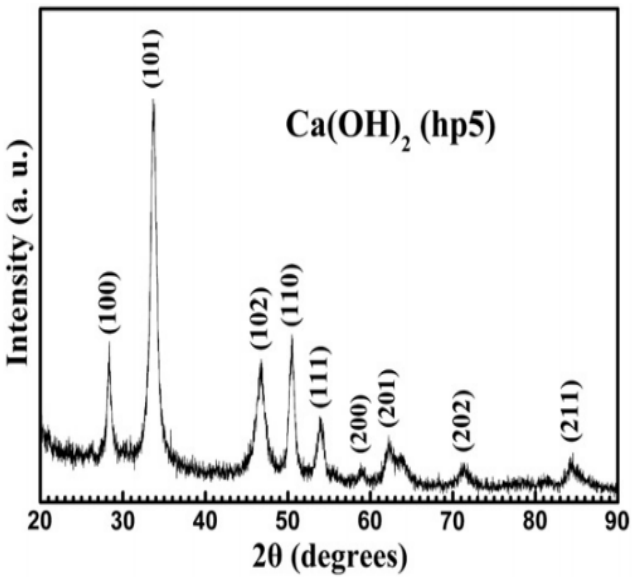

Figure 4: XRD image of Calcium hydroxide Nanoparticles

Sample preparation

Pro root MTA and Biodentine were mixed according to the manufacturer's instructions. Nanosilver, nano calcium hydroxide, nano chitosa and nano hydroxyapatite (Sigma Aldrich, USA) was added to GIC powder by 1% weight by a digital weighing machine (AND GR-200 Analytical Balance, Lab Recyclers Inc., Gaithersburg MD, USA) and then mixed with GIC liquid (11). Freshly mixed pastes were compacted into polyvinyl chloride molds (8 ± 0.1 mm diameter x 1.6 ± 0.1 mm), and the excess was removed. The exposed upper surface area of each sample was 50.24 ± 0.01 mm^2 (5).

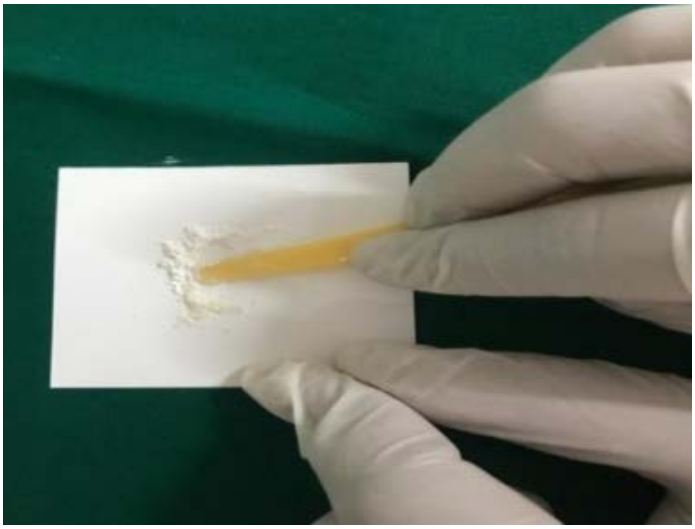

Figure 5: manipulation of cement

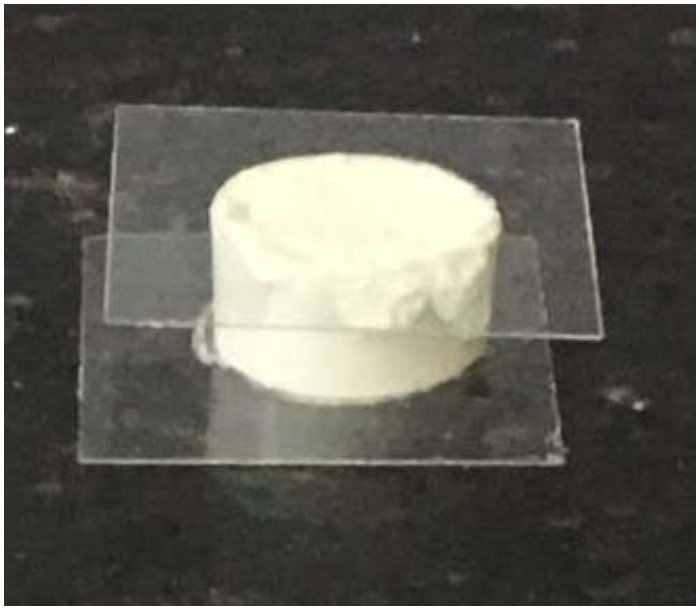

Figure 6: Freshly mixed pastes were compacted into polyvinyl chloride mould and covered with cover slip

Grouping of samples

The specimens were divided into 6 groups. namely, **Group 1:** Pro Root MTA (n=10), **Group 2:** Biodentin (n=10), **Group 3:** Nano Chitosan GIC (n=10), **Group 4:** Nano Silver GIC (n=10), **Group 5:** Nano Hydroxyapatite GIC (n=10), **Group 6:** Nano Calcium Hydroxide GIC (n=10)

Measurement of solubility and water sorption

The specimens were unmolded, dried with filter paper, and weighed (initial mass, Di). Each disk was immersed vertically in 20 mL distilled water at 37C. After 24 hours, the mass while suspended in water (S) was determined. Excess water was removed, and the saturated mass (M) was recorded. Samples were dried at 37C to a stable weight (dry mass [Df]). Each weight measurement was repeated 3 times to the nearest 0.001 g using an analytical balance (Bel Engineering series M, Monza, Italy) (5).

Calculation of water sorption and solubility

Water sorption is calculated using the formula: (A [([M-D_f])/D_f] X 100)

Solubility is calculated using the formula: (S [S = ([(D_i-D_f)]/D_f) X 100]) (5).

Calcium release

The molds were placed on the bottom of cylindric polystyrene containers (3-cm high with a 4-cm diameter) with 10 ml deionized water, sealed, and stored at 37C. The water was collected and renewed after 24 hours and 7 days. Calcium ion release was measured until the measurement stabilized.

Freshly prepared samples (approximately 5 minutes after mixing) and samples aged in SBF (simulated body fluid), prepared through Kokubo method for 24 hours and 7 days were examined ‘‘wet’’ using an environmental scanning electron microscope (ESEM; Zeiss EVO 50; Carl Zeiss, Oberkochen, Germany) connected to a secondary electron detector for energy dispersive x-ray analysis (EDX; Oxford INCA 350 EDS, Abingdon, UK). EDX provided qualitative and semi quantitative measurements of atomic calcium and phosphorous to calculate the superficial calcium to phosphorus (Ca/P) atomic ratios (5).

Results

Solubility

Table 1: solubility in wt% at 24 hours and 7 days.

	24 hours (wt%)	7 days (wt%)
Calcium hydroxide	22.12	22.88
Silver	11.74	12.74
Chitosan	25.78	26.45
Hydroxyapatite	12.36	13.12
Biodentine	11.12	12.69
ProRoot MTA	10.77	10.99

Water sorption

Table 2: water sorption in wt% at 24 hours and 7 days.

	24 hours (wt%)	7 days (wt%)
Calcium hydroxide	1.54	1.70
Silver	4.68	4.1
Chitosan	3.61	3.26
Hydroxyapatite	3.78	3.48
Biodentine	12.99	12.34
ProRoot MTA	14.13	14.33

Calcium release

Table 3: calcium release at 24 hours and 7 days.

	24 hours	7 days
Pro Root MTA	10.58	8.87
Silver	7.56	6.25
Hydroxyapatite	18.78	16.56
Calcium hydroxide	18.55	17.66
Chitosan	1.55	1.25
Biodentin	20.45	15.67

Statistical analysis

Kruskall wallis test is performed based on variables among all 6 groups. The Alternative hypothesis would be there is significant difference between 6 groups and the opposite would be null hypothesis.

Table 4: Kruskal wallis test to measure the calcium release at 24 hours

Ranks			
Calcium release at 24 hours	Group	N	Mean
	Calcium	10	25.50
	Silver	10	15.50
	Chitosan	10	41.15
	Hydroxyappatite	10	39.85
	Biodentine	10	5.50
	Pro root MTA	10	55.50
	Total	60	

Table 5: Kruskal wallis test to measure the calcium release at 7 days

R			
Calcium release at 7 days	Group	N	Mean
	Calcium	10	25.50
	Silver	10	15.50
	Chitosan	10	45.20
	Hydroxyappatite	10	55.50
	Biodentine	10	5.50
	Pro root MTA	10	35.80
	Total	60	

Table 6: Kruskal wallis test to measure the solubility at 24 hours

Ranks			
Solubility at 24 hours	Group	N	Mean
	Calcium	10	45.50
	Silver	10	16.50
	Chitosan	10	55.50
	Hydroxyappatite	10	35.50
	Biodentine	10	22.90
	Pro root MTA	10	7.10
	Total	60	

Table 7: Kruskal wallis test to measure the solubility at 7 days

Ranks			
Solubility at 7 days	Group	N	Mean
	Calcium	10	45.50
	Silver	10	20.50
	Chitosan	10	55.50
	Hydroxyappatite	10	34.00
	Biodentine	10	22.00
	Pro root MTA	10	5.50
	Total	60	

Table 8: Kruskal wallis test to measure the water sorption at 24 hours

Ranks			
Water Sorption at 24 hours	Group	N	Mean
	Calcium	10	5.50
	Silver	10	35.50
	Chitosan	10	19.95
	Hydroxyapp	10	21.05
	Biodentine	10	45.50
	Pro root	10	55.50
	Total	60	

Table 9: Kruskal wallis test to measure the solubility at 7 days

Ranks			
Water Sorption at 7 days	Group	N	Mean Rank
	Calcium	10	5.00
	Silver	10	32.00
	Chitosan	10	17.72
	Hydroxyappa	10	19.28
	Biodentine	10	41.00
	Pro root	10	50.00
	Total	60	

The above table denotes the total number of samples in all 3 groups and mean ranks denotes the ranking based on variable scores. Higher the rank higher the values, if statistically significant.

Table 10: test statistics of kruskal wallis and grouping variables

Test						
	CR	CR	Sol	Sol	WR	WR
Chi-	55.772	57.189	54.967	54.417	55.767	50.134
df	5	5	5	5	5	5
Asymp. Sig.	.000	.000	.000	.000	.000	.000
a. Kruskal Wallis Test						
b. Grouping Variable: group						

*CR= Calcium release

*Sol= Solubility

*WR=Water Sorption

P value less than 0.05 shows that all groups are significantly different from each other based on solubility, calcium release and water sorption.

Discussion

Bacteria and bacterial products are the main causes of pulpitis and apical periodontitis. The goals of root canal therapy are to eliminate microorganisms inside a root canal system and to prevent re-infection. Bacterial colonization in a complex root canal system is difficult to eradicate by routine cleaning and shaping procedures. Intracanal medicament is normally used in endodontic treatment to eliminate the remaining bacteria and prevent contamination during appointments. However, failure of non-surgical root canal treatment can occur due to persistent infection or re-infection. It appears that certain species of microorganisms, especially gram-positive facultative anaerobes, possess great resistance to antimicrobial agents used during endodontic treatment (21, 22)

The aim of periapical surgery is to remove diseased tissue and to carry out root-end resection and root-end filling to seal the communication between the periapical tissues and the root canal system (Johnson & Witherspoon 2006). The purpose of a root-end filling is to produce a hermetic seal after root-end resection (Torabinejad & Pitt Ford 1996, Kim & Kratchman 2006). Root-end filling materials are in direct contact with the periapical tissues and for this reason, an ideal material should be biocompatible, impervious to dissolution or breakdown by the tissue fluids, non resorbable, adapting as closely as possible to the dentinal walls of the root-end preparation and possess good handling characteristics (Torabinejad & Pitt Ford 1996) (21)

Gartner and Dorn proposed that an ideal root-end filling material should be easy to manipulate, radiopaque, dimensionally stable, non-absorbable, insensitive to

moisture, adhesive to dentin, nontoxic, and biocompatible. Many materials have been used for root-end fillings in endodontic surgery. However there is no one material that is universally accepted as the best (27)

Numerous materials have been suggested as root end filling materials; Gutta-percha, Amalgam, Zinc oxide-eugenol, Cavit, Composite resin, Gold foils, and Glass ionomers etc. In this study we have used nanoparticle modified Glass ionomer cement, Pro Root Mineral trioxide aggregate and Biodentine™ as root end filling materials. Glass ionomer cement is a material with universal properties. It is a dentin substitute. Its ability to bond chemically to tooth structure provides an excellent marginal seal. Studies have shown that glass ionomer cement possesses antibacterial activity due to slow release of fluoride (13). However, clinically, the plasticity and stickiness of glass-ionomer cement impede condensation into the root-end cavity, and it is extremely sensitive to moisture.

Mineral Trioxide Aggregate (MTA) has a more predictable clinical success compared with other root-end filling materials due to its lower cytotoxicity, better biocompatibility and microleakage protection (Von Arx, 2011; Hassanien et al., 2015). On the other hand, MTA has several disadvantages such as; long setting time, difficult handling, expensiveness, potential discoloration, and lower compressive and flexural strengths (Unal et al., 2010; Negm et al., 2017).

New experimental Calcium silicate based restorative cement is introduced into the market under the name of Biodentine™ (Septodont, SaintMaur-des-Fosses, France). It shares both its indications and mode of action with calcium hydroxide, but it has overcome the drawbacks of calcium hydroxide. Biodentine is considered as an alternative to MTA because it has several similar properties when compared with MTA with better consistency and faster setting time (34) Biodentine™ consists of a powder in a capsule and liquid in a pipette. The powder is mixed with the liquid in a capsule in the triturator for 30 seconds. Once mixed, Biodentine™ sets in about 12 minutes. During the setting of the cement calcium hydroxide is formed. The consistency of BiodentineTM reminds of that of phosphate cement.

Biodentine™ is a calcium silicate based material used for crown and root dentin repair treatment, repair of perforations or resorptions, apexification and root-end fillings. Biodentine is recommended as an endodontic repair material due to its good sealing ability, short setting time, high compressive strengths, biocompatibility and biomineralization properties (34).

In recent years, chitosan NPs have emerged as potential carriers for delivery of drugs ranging from small organic molecules to proteins and nucleic acids. The chitosan nanoparticles can be easily dispersed in distilled water or aqueous buffers of neutral pH and exhibit higher antibacterial efficacy in comparison to chitosan. It is a non-toxic, inexpensive, and highly biocompatible biopolymer that can be easily biodegraded through different hydrophilic enzymes, promoting positive biological effects such as bactericidal, anti-inflammatory, antioxidant, antitumor, and healing properties (14-17). Nanomaterials based on chitosan have been widely used in the regeneration of different types of tissues, especially skin and bones, and have been used in many other biomedical and pharmaceutical applications (30-33).

On the other hand, AgNPs are the most common metallic nanomaterials used for the control of several types of microorganisms due to their very well-known antimicrobial properties even in drug-resistant microorganisms, including the E. faecalis strain.

Hydroxyapatite (HA) is one of the bioceramic materials that forms the principal mineral component of bone and

comprises 60% to 70% of the calcified skeleton. HAp has been widely used in repair of hard tissues, and common uses include bone repair, bone augmentation, as well as coating of implants or acting as fillers in bone or teeth. However, the low mechanical strength of normal HAp ceramics generally restricts its use to low load-bearing applications. Recent advancements in nanoscience and nanotechnology have reignited investigation of nanoscale HAp formation in order to clearly define the small-scale properties of HAp. It has been suggested that nano HAp may be an ideal biomaterial due to its good biocompatibility and bone integration ability (12, 13).

Calcium hydroxide (CH) is widely used as an intracanal medicament. In order to be effective, the hydroxyl ions (OH-) in CH should diffuse into the dentinal tubules and accessory canals where the bacteria are harbored. The release of these ions induces an alkalizing effect and destroys the cellular membranes and protein structures. CH also dissolves the remaining tissue debris. It has the ability to promote an osteogenic environment and prevent root resorption (23-26)

Many studies used dye penetration method for the assessment of marginal adaptation and microleakage. However, traditional dye leakage evaluation has several limitations including dissolution of dye during the process and difficulty in observing the maximum penetration and it has been well documented. In the present study, SEM examination was used to determine the marginal adaptation of root-end filling materials to the surrounding tooth structure (27)

Ideal materials for sealing root-end cavities should prevent leakage. They should have dimensional stability, should adhere to the walls of the cavity, should be resistant to resorption, and should be moisture resistant; they should also be nontoxic and biocompatible to promote healing (42). The ability to release calcium ions able to diffuse through dentin and inside the surrounding tissues is a key factor for successful endodontic and pulp capping therapies because of the action of calcium on the differentiation of mineralizing cells as dental pulp cells , cementoblasts , osteoblasts, periodontal fibroblasts , mesenchymal stem cells, and hard tissue mineralization (5).

Thus, the aim of the study is to evaluate the solubility, water sorption, marginal adaptation and bioactivity of conventional and modified retrograde filling materials after immersion in simulated body fluid (SBF).

Solubility was evaluated based on the methodology by Carvalho-Junior, et al. (2007), with samples of smaller dimensions than those established by ISO 6876/200217 or ANSI/ADA Standard No. 571. According to these standards, Solubility is evaluated by the difference in mass in grams, between before and after immersion in water, and has limitations that may influence the result. Solubility of a solid material is defined as the amount of a substance dissolved in a solvent. The ISO test measures the elution of a water-soluble material, since the material may present degradation during storage or water absorption (29)

The results of the study show that, Chitosan had the highest calcium release and biodentin had the lowest calcium release after 24 hours. Hydroxyappatite had the highest calcium release and biodentin had the lowest calcium release after 7 days which could be due to the principal mineral component of bone and comprises 60% to 70% of the calcified skeleton (35)

However, a study conducted by Laurent P et al., assessed the ability of Biodentine, MTA, calcium hydroxide and Xeno III adhesive resin to induce reparative dentin synthesis and transforming growth factor beta 1 (TGF- b1) secretions. They showed that both Bodentine and MTA involved in early odontoblastic differentiation and

initiation of mineralisation and thus form reparative dentin synthesis then other two materials (35).

Chitosan had the highest solubility and pro root MTA had the lowest solubility after 24 hours and after 7 days. Several studies that had been conducted to assess the solubility of MTA and concluded that, with increase in water-to-powder ratio, release of calcium from MTA increases which accelerates its solubility (36, 37, 45).

Pro root MTA had the highest water sorption and Calcium hydroxide had the lowest water sorption after 24hours and 7days. Koubi, et al.20 (2012) evaluated the marginal integrity of restorations by using Biodentine, and related that the reduced size of the calcium silicate cement particles and small expansion of the material could contribute to its greater filling ability. Dawood, et al. (2014) investigated the physical properties of Biodentine and MTA Angelus, and observed greater solubility for Biodentine after 7 days.

Singh, et al. (2015) compared the solubility of Biodentine and MTA at the time intervals of 24 hours, 3, 10, 30 and 60 days, and demonstrated that Biodentine showed greater solubility at the time intervals of 30 and 60 days. Kaup, et al. (2015) evaluated the solubility of Biodentine and MTA ProRoot and found that Biodentine showed greater solubility after 28 days, indicating a mass loss of 4.610 (±1.402) % (28)

A SEM study conducted on sealing ability of Biodentin, MTA and GIC to dentin concluded that Biodentin exhibited better marginal adaptation to dentin in comparison to MTA and GIC cements and also highlighted the influence of time on marginal adaptation (28).

There is very limited literature available on the use of calcium hydroxide and hydroxyapatite nanoparticles as a retrograde filling material. However, the limitations of the current study include, microleakage should have also been assessed and also, longer follow up periods should be undertaken to assess the solubility, water sorption and bioactivity of the materials.

Clinical relevance

The clinical significance of the study is that, an ideal retro-grade filling material should prevent leakage, have dimensional stability, adhere to the walls of the cavity, resistant to resorption, and should be moisture resistant; they should also be nontoxic and biocompatible to promote healing (42). The ability to release calcium ions able to diffuse through dentin and inside the surrounding tissues is a key factor for successful endodontic and pulp capping therapies because of the action of calcium on the differentiation of mineralizing cells as dental pulp cells , cementoblasts , osteoblasts, periodontal fibroblasts , mesenchymal stem cells, and hard tissue mineralization (5).

Conclusion

Chitosan had the highest calcium release and biodentin had the lowest calcium release after 24 hours. Hydroxyapatite had the highest calcium release and biodentin had the lowest calcium release after 7 days. Chitosan had the highest solubility and pro root MTA had the lowest solubility after 24 hours. Chitosan had the highest solubility and pro root MTA had the lowest solubility after 7 days. Pro root MTA had the highest water sorption and Calcium hydroxide had the lowest water sorption after 24hours. Pro root MTA had the highest water sorption and Calcium hydroxide had the lowest water sorption after 7days.

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