CHARACTERIZATION OF A NEW BIOACTIVE CALCIUM SILICATE CEMENT - AN IN-*VITRO* STUDY

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in partial fulfilment for the degree of

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BRANCH – IV

DEPARTMENT OF CONSERVATIVE DENTISTRY AND ENDODONTICS

2017 – 2020

CERTIFICATE

This is to certify that the Dissertation entitled **"**CHARACTERIZATION OF A NEW BIOACTIVE CALCIUM SILICATE CEMENT- AN IN-*VITRO* STUDY**"** by **Dr. ALMAS BEGUM. A,** Post Graduate student, **MDS Conservative Dentistry and Endodontics**, Madha Dental College & Hospital, Chennai – 69 Submitted to Tamilnadu Dr. M.G.R. Medical University for the MDS Degree Examination May 2020 is a bonafide research work carried out by her under my supervision and guidance.

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CERTIFICATE - II PLAGIARISM

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DECLARATION OF THE CANDIDATE

I, **Dr. ALMAS BEGUM. A,** hereby declare that this dissertation titled **"CHARACTERIZATION OF A NEW BIOACTIVE CALCIUM SILICATE CEMENT- AN IN-*VITRO* STUDY"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. V. SUSILA ANAND, MDS, PhD. Professor & Head,** Department of Conservative Dentistry & Endodontics, Madha Dental College and Hospital, Chennai - 600069**.**

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Ref No : MDCH/IEC/2018/03

DATE : 30.03.2018

(Sub :IEC review of the research proposals)

Title of the work : CHARACTERIZATION OF A NEW BIOACTIVE CALCIUM SILICATE CEMENT - AN INVITRO STUDY

Principal investigator: Dr. A, ALMAS BEGUM, Ist YEAR MDS

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The request for approval from the Institutional Ethical Committee (IEC) considered at the Institutional Ethics Committee meeting held on the 30.03.2018 , at Madha Dental College and the documents related to the study referred above were discussed and reported to us through your letter dated 23.2.18 have been reviewed. The decision of the members of the committee, the secretary and the Chairperson IEC of Madha Dental College is here under:

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LIST OF ABBREVIATIONS

PC	Portland cement
nm	Nanometer
mm	Millimeter
μm	Micrometer
CLG	Calcium Lactate Gluconate
MTA	Mineral Trioxide Aggregate
WMTA	White Mineral Trioxide Aggregate
GMTA	Gray Mineral Trioxide Aggregate
$CaCl_2$	Calcium chloride
MPa	MegaPascals
e.g.	Example
$CaCO_3$	Calcium carbonate
Na_2HPO_4	Disodium phosphate
CSC	Calcium Silicate Cement
CPP ACP	Casein Phospho Peptide-Amorphous Calcium Phosphate
CS	Calcium silicate-based cement
%	Percentage
≤	Less than or equal to
≥	Greater than or equal to
~	Similar to

ZrO_2	Zirconium oxide
Zn	Zinc
Ca	Calcium
BO	Bismuth oxide
HA	Hydroxyapatite
PBS	Phosphate Buffer Solution
EDTA	Ethylene Diamine Tetraacetic Acid
P value	Probability value
PA	Peracetic acid
CA	Citric acid
SEM	Scanning Electron Microscopy
EDX/EDAX	Energy Dispersive Xray Analysis
XRD	X-Ray Diffraction analysis

INTRODUCTION

INTRODUCTION

Bioactivity and biomimetics are gaining popularity in dentistry in general, and in Conservative Dentistry and Endodontics in particular. These materials stimulate native tissues to repair or regenerate the appropriate tissue where disease and inflammation has caused an imbalance or destruction. Caries, resorption, fracture and other idiopathic diseases cause destruction of dental tissues like dentine, cementum, periodontal ligament and periapical bone. Selecting an ideal bioactive cement for such treatments is paramount in ensuring success.

A bioactive material is capable of inducing a biological activity i.e, (1) indicating calcium ions release, calcium hydroxide production, electro conductivity, formation of an interfacial layer between the dentinal wall & cement and apatite crystals formation over the surface of the material even in a synthetic tissue fluid environment such as phosphate buffered saline. (2) Bioactivity of a ceramic material is a surface property that provides a bond between the material and living tissues without fibrous encapsulation. The most important part of this mechanism is the ability to re-mineralize calcium phosphates on a material's surface in vivo. (3) Invitro bioactivity is formation of hydoxyapatie (HA) on the surface when in contact with simulated body fluid (SBF). (4) Hence it serves as a standard benchmark.

The primary components are tricalcium silicate and dicalcium silicate. These ceramic powders are the similar compounds as present in commercial Portland cement utilized for construction. These unique compounds react with water in room temperature, called as a hydraulic setting reaction, to be used as a solid mass. Being moisture tolerant (hydrophilic, hygroscopic) is a great advantage where moist tissues can hinder with material placement and setting. (5)

The first reference to bioactive cements came from Dr. Witte, during 19th century who mixed Portland cement with water, carbolic acid or creosote for placing underneath a gold filling. In 1993, same cement was reconsidered for use by Dr. Torabinejad and Mr. White by patenting the use of cement in Endodontics (Patents 5415547 and 5769638). The Mineral Trioxide Aggregate (MTA) was regarded by Torabinejad et al. as a potent material to seal off pathways between the pulpal and periodontal tissues. (6) In 20th century, an article on hydraulic ceramics introduced the experimental material as ‘‘MT aggregate”. The MTA material was referred as a hydrophilic powder composed of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide and a few other mineral oxides. In the first patent it was named as a Portland cement ceramic powder composed of dicalcium silicate ($2CaO.SiO_2$), tricalcium silicate ($3CaO.SiO_2$), tricalcium aluminate ($3CaO.Al_2O_3$) and tetracalcium aluminoferrite ($4CaO.Al_2O_3.Fe_2O_3$)”. (5) (7) It is formed of naturally occurring minerals used to create tri/ dicalcium silicates. The trioxides in cements refer to the oxides commonly used in describing Portland cement: calcia, silica and alumina. The aluminum oxide (alumina) is common in construction-grade Portland cement because of alumina’s concurrence with calcium and silicate minerals, although alumina is not an essential component to create a hydraulic tri/dicalcium silicate powder. The designation ‘‘trioxide” is a misnomer since other oxides were present in the original experimental material in quantities greater than 1%, including iron oxide and bismuth oxide. ‘‘Aggregate” may denote the addition of radiopaque component, like bismuth oxide, analogous to addition of sand or gravel to make concrete. Alternatively, it may refer to the aggregation of the tricalcium silicate, dicalcium silicate, and tricalcium aluminate crystals in the powdered cement. Conversely, abbreviations like hydraulic calcium silicate cements (hCSCs) or tricalcium silicate (TCS) for the same MTA-type materials are used in the literature. (5)

Gypsum and tetracalcium aluminoferrite are important determinants of setting time. MTA contains approximately half the gypsum content of Portland cement and smaller amounts of aluminum, which provides a longer working time than Portland cement. Although Portland cement could serve as an MTA substitute, it is essential to emphasize Portland cement (PC) and MTA are not identical materials. (8)

MTA has been successfully used for several endodontic procedures such as pulp capping, apexogenesis,, root resorption, perforation repair etc. (9) because of its advantages:

- Bacteriostatic- due to high alkaline pH,
- Induce regeneration of dentin, cementum and bone,
- Excellent sealing ability,
- Sets even in the presence of moisture

Though MTA is so widely used due its favourable properties, there does exist few shortcomings. They are

- Longer setting time : MTA has a longer setting time of 2 ½ to 3 hours
- Presence of toxic elements in the material (Bi)
- Poor handling properties
- Absence of a known solvent, and difficulty of removal after cure
- Cost
- Discolouration

Presence of transitional elements namely iron, manganese, copper and chromium imparts strong color to the material in it oxide forms. Bismuth causes discoloration due to its yellow oxide.

The fact that MTA is essentially PC with bismuth oxide has encouraged studies aimed at using PC as an alternative to MTA. MTA and PC have similar composition and biological properties however with different chemical and physical properties. MTA contains fewer toxic heavy metals, chromophores, aluminium and potassium species. Either MTA is made from different raw materials compared with normal PC, or the raw materials used for MTA are purified during the production process. PC is composed of 4 main oxides, namely lime (CaO), silica (SiO_2), alumina (Al_2O_3), and ferric oxide (Fe_2O_3). The lime is obtained by the decomposition of limestone (CaCO3), and the other components are produced from shale. Arsenic and lead are impurities in limestone, which is used to manufacture PC. High concentrations of arsenic have been found in cement dust, and alkaline by-products are formed during cement manufacturing. Therefore, PC is not widely accepted for clinical use. (7)

Currently, the choice of a specific material for endodontic use should not rely solely on biological and functional aspects but also on the esthetic considerations. Thus, biomaterials used in Endodontics should be chromatically stable, present optical properties similar to dental structures, and exhibit no staining effects of hard dental tissues over time. However, based on clinical observations, during preparation, many products, even light- colored products, change color within a short time after application and when they come in contact with other substances (eg, sodium hypochlorite [NaOCl], which is routinely used during endodontic treatments). In addition to the color change in the biomaterial, changes may occur in the color of the tooth tissue; this problem limits the use of a biomaterial in anterior teeth. (10)

A case report by Bortoluzzi et al showed that teeth restored with grey MTA also showed darkening of the facial marginal gingiva in addition to the crown discoloration after 6 months of its placement. (11) One possible mechanism of tooth discoloration by MTA is related to the oxidation of the iron content of the set material, which is attributed to the calcium aluminoferrite phase of the powder. (12) Also materials containing bismuth oxide had high staining potential. When bismuth oxide interacted with collagen, it was converted to a black precipitate. The cause of discoloration is still debatable; however, the interaction of bismuth oxide with collagen present in tooth tissue and sodium hypochlorite, which is routinely used during root canal therapy, has been indicated as the main causative factor. (13)

However, newer materials that contained zirconium oxide as a contrasting substance did not have high staining potential. Materials based on tricalcium silicate with alternative radiopacifiers are available clinically. Such formulations include BioDentine, Retro MTA, Portland cement, EndoSequence Root Repair Material, Odontocem, MM-MTA, Neo MTA Plus, MTA Ledermix and BioAggregate, which do not include bismuth oxide in their formulation. (10) (14)

MTA and any material used for the treatment of immature permanent teeth should produce calcium hydroxide because this is a necessary prerequisite for treatment success. The hydration reaction of tricalcium silicate results in the formation of calcium hydroxide. However, not all materials based on tricalcium silicate produce calcium hydroxide on hydration. The hydration of the materials resulted in the formation of calcium hydroxide in the early stages of the reaction and also exhibited the potential to form calcium phosphate in contact with tissue fluids,

thus showing high reactivity. The lack of calcium hydroxide as the material ages may cause a reduction in material bioactivity and biocompatibility in the long-term. (15) Material additives like calcium phosphate found in BioAggregate reduce the free calcium hydroxide available. The addition of resins to the materials as in Theracal also reduces the free calcium hydroxide, making these materials potentially unsuitable in the treatment of immature permanent teeth. Bismuth oxide is replaced by zirconium oxide in BioDentine and tantalum oxide in Neo MTA Plus and BioAggregate. These alternative radiopacifers were adequate because both materials produced comparable radiopacity, and they did not interfere with the hydration of the materials. (13)

However Garcia et al evaluated the potential of EndoBinder for staining teeth, with or without a radiopacifying agent, in comparison with MTA. The results showed that the staining caused by MTA was not related to bismuth oxide because EndoBinder presented similar staining in the presence or absence of a radiopacifying agent. So presence of Aluminium in the EndoBinder was suggested as the cause of discoloration. (14)

To overcome these shortcomings – a non-Iron, non-Aluminium and non-Bismuth containing calcium silicate cement was formulated which is an indigenous production and hence is cost effective.

AIMS AND OBJECTIVES

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Aims and objective of this thesis are:

To formulate and characterize an indigenous affordable new Experimental Calcium Silicate cement to overcome the disadvantages of MTA and other contemporary Calcium Silicate cements.

The null hypothesis is: There will not be any significant difference between the new Experimental Calcium Silicate cement and MTA in terms of setting time, compressive strength, push-out bond strength, color stability and bioactivity.

The alternate hypothesis is: There will be a significant difference between the new Experimental Calcium Silicate cement and MTA in terms of setting time, compressive strength, push-out bond strength, color stability and bioactivity.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The term "bioactive material" was coined by Dr. Larry Hench in 1969. He was looking for an improved graft material for bone reconstruction in injured soldiers returning from Vietnam War. He was searching for an ideal material that could bond with living tissues without being rejected. Bioglass (calcium silicophosphate glass) was thus developed as a completely synthetic material that chemically bonds with bone. Hench defined bioactive material as "one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material." (16)

However, the first ever bioactive material known to medical field – Portland cement which was developed in the year 1824 by Joseph Aspdin is a predecessor for most of the Calcium Silicate based cements used nowadays. The first ever used calcium silicate, Portland cement dates back to Roman times when a cement able to set in water was made by grinding lime and a volcanic product found at Puteoli (hence called *pozzolana*) around Neapolis. Roman concrete set quickly when Pozzolana was mixed, even when submerged in water, allowing the construction of bridges, monuments and buildings. During the middle ages the secret of this cement was lost. Again in the 18th century, an English engineer, John Smeaton, rediscovered the correct proportions of the cement using clay and limestone. In 1824, an English brick-layer, Joseph Aspdin, patented a process for making what he called Portland cement. The first great bridge in the USA built in the late 19th century was made of Portland–Pozzolanic cements. Modern Portland cement is made by mixing lime, alumina, silica, and iron oxide and heating the mixture until it almost fuses. Pozzolana still remains the main component of many Portland cements. (17) Later in the year 1878, Dr. Witte in Germany was the first to use Portland cement in dentistry to fill root canals. (18)

Mineral trioxide aggregate (MTA) was developed at Loma Linda University (Loma Linda, California, USA) and was first described in the dental scientific literature in 1993. Mineral trioxide (MT) aggregate, was developed to seal off all of the pathways of communication between the root canal system and the external surface of the tooth. The principal compounds present in the mineral trioxide material are tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide. It was suggested as a potential root-end filling material. (19) It received US Food and Drug Administration approval in 1998.

The first commercially available product was gray mineral trioxide aggregate (GMTA), marketed as **ProRoot** MTA (DENTSPLY-Tulsa Dental). To decrease the potential for tooth discoloration observed when GMTA is used in anterior teeth, an alternative formulation, known as tooth-colored MTA or white mineral trioxide aggregate (WMTA) (DENTSPLY), was developed in 2002. The composition of WMTA is almost identical to that of GMTA, except for the absence of iron compound (tetracalcium aluminoferrite). It contains 54.9% less aluminium oxide, 56.5% less magnesium oxide, and 90.8% less ferrous oxide as compared with GMTA. Thus, reduction in the iron compound (ferrous oxide) is most likely the main reason for the lighter color change, with reduction in magnesium acting as a contributing factor. Both products have demonstrated similar physicochemical and biologic properties, differing mainly in chemical composition. GMTA has particle sizes of 1 µm to 10 µm and exhibits significantly higher initial and final setting times, greater compressive strength, and less microleakage (as an apical barrier) as compared with white mineral trioxide aggregate (WMTA)

In 2004, MTA-Angelus (Angelus Indústria de Produtos Odontológicos) was introduced and was available as grey (AGMTA) and white (AWMTA). It is a silicate cement mainly consisting of 80% PC and 20% bismuth oxide. It contains a lower content of bismuth oxide and

magnesium phosphate and a greater amount of calcium carbonate, calcium silicate, and barium zinc phosphate than GMTA. As opposed to GMTA, it shows presence of aluminum and has no iron. (20)

In 2006, an experimental material called **calcium enriched mixture (CEM)** cement was formulated using different calcium compounds like calcium salt, calcium oxide, calcium silicate, and calcium phosphate compounds. It had good sealing property, handling characteristics and chemical properties. The mixed cement was not sticky and it did not adhere to applicator and hence could be condensed it easily. (21)

In 2007 a new formulation of an endodontic material named **EndoBinder** was developed at the Federal University of São Carlos and was composed of calcium aluminate cement plus additives. It was conceived to preserve the properties and clinical applications of MTA. It is composed of Al_2O_3 (68 wt%) and CaO (31 wt%). The additives used were a 0.6 wt% polymeric dispersant of the polyglycol family; 2.8 wt% $CaCl_2$ to induce plasticity into the cement; and 25 wt% ZnO to provide radiopacity. (22)

Suh et al in 2008 introduced new light-cured resin-modified calcium silicate-filled base/ liner material - **TheraCal** (Bisco Inc, Schamburg, IL, USA) designed for direct and indirect pulp capping containing approximately 40% wt mineral material (type III Portland cement), 10% wt radiopaque component, 5% wt hydrophilic thickening agent (fumed silica) and approximately 45% resin. It was shown to have the ability to release calcium ions and create an environmental pH close to physiological pH after 7 days which favours the formation of apatite and induce the differentiation of odontoblasts to form new dentine. Its ability to polymerize to a depth of 1.7 mm may avoid the risk of untimely dissolution. (23)

Zhang et al in 2009 investigated the apical sealing ability of a newly introduced root canal sealer, **iRoot SP** Root Canal Sealer and found that it was equivalent to AH Plus sealer in apical sealing ability. iRoot SP is a convenient, premixed, ready-to-use injectable white hydraulic cement paste developed for permanent root canal filling and sealing applications. It is insoluble, radiopaque, and aluminum-free material based on a calcium silicate composition, which requires the presence of water to set and harden. It is composed of biocompatible and nontoxic materials that include calcium phosphate, calcium silicates, zirconium oxide, and calcium hydroxide. It can form a hermetic seal inside the root canal and be used for filling root canals with or without gutta-percha points. Superior qualities and handling abilities make iRoot SP an innovative novel root canal sealer. (24)

In 2009, **BioAggregate (BA)** is a new retrograde filling and root canal perforation repair material. It is composed of tricalcium silicate, dicalcium silicate, tantalum pentoxide, and monobasic calcium phosphate. Tantalum pentoxide in BA provides radiopacity instead of bismuth oxide in MTA, and monobasic calcium phosphate in BA adjusts its hydrate setting. BA and MTA have comparable, strong antimicrobial activity against *E. faecalis*. (25)

BioDentine was developed by Septodont's Research Group in 2010 as a new class of dental material with good mechanical properties, excellent biocompatibility, as well as a bioactivity. It is suitable as a dentine replacement material whenever original dentine is damaged. In addition to the chemical composition based on the Ca_3SiO_5 – water chemistry which brings the high biocompatibility of already known endodontic repair cements (MTA based), Septodont increased the physico-chemical properties which make BioDentine clinically easy to handle and

compatible. Sealing ability of this biomaterial was also assessed to be equivalent to glass-ionomers, without requiring any specific conditioning of the dentine surface. (26)

In 2010, **Capasio** Powder and liquid use alternative calcium cements and novel setting reactions for a shorter working time and greater resistance to acid. Capasio is composed of bismuth oxide, dental glass, and calcium aluminosilicate with a water-based gel. The material is slightly less basic than white MTA upon the final setting, has a setting time of 9 minutes, penetrates dentinal tubules, and shows improved acid resistance and washout resistance. (27)

However, in 2012, Capasio powder was refined and renamed as **Quick-Set** (Primus Consulting), and the cationic surfactant was removed from the liquid gel component, which was thought to interfere with cell cytocompatibility. The quick setting, fine particles, high radiopacity, and slightly elevated pH properties of Capasio were however preserved in Quick-Set. (28)

In 2011, Brasseler USA (Savannah, GA) introduced the **EndoSequence Root Repair Material** (RRM) and EndoSequence Root Repair Putty (RRP), which use bioceramic technology to address some of the inconsistencies associated with conventional MTA. Bioceramics refers to the combination of calcium silicate and calcium phosphate applicable for biomedical or dental use. These new materials are produced as a premixed product. The bioceramic material is produced with nanosphere particles that allow the material to enter into the dentinal tubules and interact with the moisture in the dentin. This creates a mechanical bond on setting. (29)

In 2011, **Ortho MTA** (BioMTA, Seoul, Republic of Korea) was introduced for apex closure of an immature root, orthograde root canal filling, perforation repair, and retrograde filling. OrthoMTA consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, free calcium oxide, and bismuth oxide. There is absence of arsenic (As) and the less amount of chromium (Cr) in OrthoMTA than in ProRoot MTA. (30)

In 2012, a new MTA root canal sealer was introduced in the market, **MTA Fillapex**, to be used in root canal filling. However, when analyzing the chemical composition, it is worth emphasizing that this is a resin cement containing MTA. For that reason, it was expected that Fillapex MTA acted as a repairer. The biocompatibility of MTA repairer (White MTA) and MTA sealer (Fillapex MTA) was assessed by the MTT cytotoxicity test. But MTA Fillapex sealer showed the highest cytotoxicity. (31)

In 2012 **Tech Biosealer Endo** (TBE, Isasan, Rovello Porro, Italy) is a endodontic sealer based on calcium silicate. Tech Biosealer is an MTA-like material available as Tech Biosealer Endo, Tech Biosealer Root End and Tech Biosealer Capping. (32)

In 2012, **Tech Biosealer root end** was used as a root end filling material in a case of Atypical facial pain related to apical fenestration and overfilling which resulted in a favourable outcome. (33)

In 2014 Tech Biosealer consists of 4 different formulations for specific clinical uses. **Tech Biosealer capping** is a di-calcium and tricalcium silicate powder containing calcium chloride, calcium sulphate and montmorillonite (is a phyllosilicate mineral derived from deposits of weathered volcanic ash characterized by high and irreversible swelling capacity owing to water adsorption.) as stabilizing and plasticizing agent, packaged in unsealed 2-piece capsules. (34)

In 2013, another MTA was introduced in the market. According to the manufacturer, **MTA Plus** (Avalon Biomed Inc. Bradenton, FL, USA) is similar in composition to ProRoot and MTA Angelus but is ground finer. MTA Plus is provided with either a gel or water ampules for mixing. MTA Plus in direct contact with fluids exhibited partial decalcification of calcium silicate hydrate in contact with the solution, microcracking and leaching of calcium hydroxide. (35)

Choi et al in 2013 evaluated the biological effects of a newly developed fast-setting, MTA– derived pozzolan cement (**Endocem)**. Endocem may be a substitute for ProRoot as an available retrograde filling material because of its short setting time and resistance to washout.(36) Later in **2015 Endocem Zr,** a zirconium oxide-enriched calcium silicate-based cement and its high Zr content (57.49 wt%) was demonstrated. Zirconium oxide has been employed as a radiopacifier as a substitute to bismuth oxide, because bismuth oxide retards the setting reaction of MTA. (37)

In 2013, **MM-MTA (**Micro Mega, Besanc¸on, France), a retrograde MTA- containing material was launched in capsule form to be used after mixing by a high frequency mixer. One unique characteristic of the product is the addition of $CaCO_3$, which reduces working time. The capsule form not only causes a cost-effective application but also facilitates the formation of a homogeneous mixture that is difficult to achieve by hand instruments. (38)

In 2014, a new type of BioMTA (Meta Biomed Co., LTD, Seoul, Korea) **– Retro MTA** was introduced to the market. Bio MTA is manufactured in both forms of OrthoMTA and RetroMTA. RetroMTA is composed of calcium carbonate, silicon dioxide, aluminum oxide and zirconium oxide. (39)

In 2015, another such material, which has a similar formula to MTA, **Neo MTA Plus** (Avalon Biomed Inc, Bradenton, FL) was introduced. This material was specifically marketed for use in pulpotomies because it does not stain the tooth structure. Bismuth oxide is replaced by tantalum oxide in Neo MTA Plus. These alternative radiopacifers were adequate because both materials produced comparable radiopacity, and they did not interfere with the hydration of the materials. (13)

In 2015, an experimental **tricalcium silicate (TCS)** based cement, being referred as **TCS 50**, was developed. The powder of TCS 50 consists of 50 wt% TCS and 50 wt% zirconium oxide; 1 M $CaCl_2$ was employed as liquid to accelerate the setting process. The powder and liquid should be mixed at a weight ratio of 3:1(40)

Recently, in 2019, a new calcium silicate-based cement**, Bio-C Pulpo** (Angelus Industry, Londrina, Brazil), was developed with the aim of overcoming some undesirable characteristics of materials already in the market. It is composed of powder: calcium silicate (tricalcium silicate (Ca_3SiO_5) and dicalcium silicate (Ca_2SiO_4)), calcium hydroxide, zirconium oxide, calcium aluminate, calcium fluoride, silicon dioxide, and iron oxide and liquid: distilled water, calcium chloride, plasticizing material and methylparaben. The plasticizing material provides a higher plasticity, improving handling and insertion, which may facilitate use in clinical procedures. Bio- C Pulpo cement exhibit characteristic of "modelling putty" after mixing. (41)

One of the main disadvantages of using MTA is its extended setting time and difficult handling. In industry, the setting time of Portland cement is controlled by grinding the cement with gypsum at the end stage of the manufacturing process. The gypsum is added by cement manufacturers to retard the setting time of the cement clinker. Removal of gypsum results in a flashset which can be controlled by the use of a super plasticising admixture which makes the material more workable. In 2005, the setting time of Portland cement was reduced by excluding the gypsum in the final stage of the manufacturing process. (42)

Addition of calcium compounds (calcium salt, calcium oxide, etc.,) in calcium enriched mixture (CEM) cement was found to reduce the setting time. (21)

There are several ways that the setting time of PC can be accelerated, thereby increasing the initial strength and decreasing early washout. In 2007, chemicals such as $CaCl_2$ were added to reduce setting time. The addition of accelerators increases the rate of hydration, thereby accelerating the setting reaction. The addition of both 1% and 2% MethylCellulose/ $CaCl_2$ combinations significantly shortened the setting time, with the 1% being significantly shorter than the 2%. Greater than 2% $CaCl_2$ adversely affects the cement by increasing the risk of drying shrinkage and reducing ultimate strength. $CaCl_2$ is partially consumed during hydration, reacting with tricalcium aluminate and forming chloroaluminate. (43) Also various accelerators such as calcium nitrite/nitrate, and calcium formate were added to GMTA and WMTA as well as to Portland cement (PC) and was found that they significantly decreased the initial setting time of PC, WMTA, and GMTA. (44)

Harrington added 10% and 15% of $CaCl_2$ to Portland cement and observed a reduction above 50% for both initial and final setting times. Kogan et al used $CaCl_2$ at 5% as a vehicle for MTA and found that it reduced to 50%, which are the same results observed by Wiltbank et al. (44) Ber et al (43) showed that adding 2% of $CaCl_2$ to Portland cement resulted in more than 50% decrease on the setting time. However in 2009, $CaCl_2$ was added to the powder rather than to the liquid, and there was a significant reduction in the setting time. (45)

In 2010, alternative calcium cements were added in **Capasio** Powder and liquid with novel setting reactions for a shorter working time and greater resistance to acid with a setting time of 9 minutes. (27)

In 2011, the hydration of Portland cement in the presence of citric acid was investigated. The results suggest that 0.1% citric acid accelerated hydration, whereas a critic acid dose >0.1% retarded hydration. This is related to the crystallization of calcium hydroxide, electrical

conductivity, concentration and mobility of the charge carriers. The addition of a Calcium Lactate Gluconate (CLG) solution to cement can supply adequate calcium ions to accelerate the setting of cement. This reduces the free water within the cement to the degree that the concentration exceeds the solubility limit of the CLG solution, causing amorphous CLG to precipitate and behave as a plasticizer. This characteristic of MTA mixed with a CLG solution improves the setting property of MTA. (46) In 2013, $CaCO_3$ was added to **MM-MTA** which reduced its working time. (38)

In 2013, Endocem, was developed by using small particles of pozzolan cement without any chemical accelerators. A pozzolan is a siliceous or siliceous and aluminous material that possesses little or no cementitious value in itself but in finely divided form and in the presence of water will react chemically with calcium hydroxide at ordinary temperature to form compounds possessing cementitious properties. The pozzolanic reaction progresses like an acid-base reaction of lime and alkalies with oxides ($SiO_2 + A1_2O_3 + Fe_2O_3$) of the pozzolan. Two things happen. First, there is a gradual decrease in the amount of free calcium hydroxide with time. Calcium hydroxide has adverse effects on the mechanical properties and durability of material. Second, during this reaction there is an increase in formation of calcium silicate hydrate and calcium aluminate hydrate, stable crystals that are so effective on the strength of material. Furthermore, the use of small particles increases the surface contact of the particles with the mixing liquid and provides rapid setting and ease of handling. The initial setting time of Endocem is 2 minutes 30 seconds, and the final setting time is 4 minutes 30 seconds. (36)

In 2015, Na_2HPO_4 was used as an accelerator to reduce the setting time of MTA to 31.60 ± 2.91 min. Na_2HPO_4 solution can be used as a cement liquid to accelerate the setting of the cement prepared from tricalcium phosphate as a cement powder. Phosphate increases the rate of hydroxyapatite formation. Strong ionic interactions between phosphate and calcium ions lead to

the formation of calcium silicate hydrate phase. (47)

Several attempts to create fast-setting MTA or its derivatives have been performed, mainly by the inclusion of chemical setting accelerators in MTA's compositions. However, the addition of such components may impair MTA's biological and physicochemical properties, but the final result remains inefficient in developing an advantageous clinical setting time. (48)

To prevent color changes, there are two alternatives. The first one is the replacement of bismuth oxide with calcium tungstate or with zirconium oxide. These substances do not cause color changes. The second alternative is to associate 5% zinc oxide with MTA. Zinc oxide prevents the change in color caused by conversion of bismuth oxide to bismite. (49)

To decrease the potential for tooth discoloration observed when GMTA is used in anterior teeth, an alternative formulation, known as tooth-colored MTA or white mineral trioxide aggregate (WMTA), was developed with the composition almost identical to that of GMTA, except for the absence of iron compound (tetracalcium aluminoferrite), which is the most likely reason for the lighter color change, with reduction in magnesium acting as a contributing factor. (20)

In 2014, **Retro MTA** which was composed of calcium carbonate, silicon dioxide, aluminum oxide and zirconium oxide was shown to have a good color stability. (39)

In 2015, **Neo MTA Plus,** in which bismuth oxide was replaced by tantalum oxide did not show any discoloration, and no phase changes were observed for the materials. (13)

Later in **2015,** Endocem Zr, a zirconium oxide-containing white MTA-like material demonstrated less staining potential due its high Zr content (57.49 wt%). (37)

Marconyak et al in 2016 found that BioDentine and ERRM did not cause significant discoloration within 60 days after application compared with the control group and concluded that bismuth oxide was a significant contributor to tooth discoloration. (50) BioDentine had a great potential for tooth staining after 1 year. They also suggested that the discoloration of BioDentine could take place in the period between 6 months and 1 year, but other authors showed that BioDentine did not cause significant discoloration. (10)

In 2017, addition of 5% ZnO addition was found to be sufficient to prevent the dental discoloration observed with MTA Angelus. It is postulated that the zinc molecules interact with the bismuth oxide, stabilizing it from phase changes when in the presence of strong oxidizing agents (51)

MATERIALS AND METHODS

MATERIALS AND METHODS

Materials:

Mineral Trioxide Aggregate - MTA Forte (Globus Medisys, India) (Fig 1)

Composition:

Tricalcium silicate, Dicalcium silicate, tricalcium aluminate – 75%

Bismuth oxide - <25%

New experimental Calcium Silicate based cement

Formulation: An experimental Calcium Silicate cement was formulated with the following composition

Calcium silicate – 40%

Calcium hydroxide – 25%

Zirconium dioxide – 10%

Zinc oxide – 5%

Strontium chloride – 15%

Calcium chloride – 5%

The above raw materials of analytical grade were procured from Merck, Germany. And the above mentioned proportions were weighed in an analytical weighing machine (Shimadzu electronic balance ELB300) with an accuracy of ± 0.01g and were mixed using an ultrasonic vibrator (Digital Ultrasonic Cleaner) and sintered in vacuum at 1200°C in preheated furnace

(Zirkonzahn furnace) at 400°C; 5°C/min rate of increase of temperature and holding at the final temperature for 2 hours. The sintered formulation was ball milled to a particle size of 2 – 3 μm.

Methods:

Mixing of cements:

MTA and new experimental Calcium Silicate cement were mixed in a water / powder ratio of 3: 1. Spatulation was done on a glass slab with a cement spatula to incorporate all the particles into the mixture and obtain a homogenous mix.

X- Ray Diffraction: (Fig 2)

The cements were mixed at a water/ powder ratio of 0.33. The cement pastes were crushed using a mortar and pestle prior to testing. Phase analysis was carried out on the cements using X-ray diffraction (XRD). The diffractometer used Cu Ka radiation at 40 mA and 45 kV. Samples were presented in powder form, and the detector was set to rotate between 5 and 45°, a sampling width of 0.05° and scan speed of 2° per minute. Phase identification was accomplished using a search-match software utilizing ICDD database (International Centre for Diffraction Data, Newtown Square, PA, USA).

Setting time: (Fig 3 & 4)

To determine the setting time, five samples from each cement were prepared. Molds for sample preparation were of 2 mm height and 10 mm width. The cements were mixed and transferred into the molds which were supported by glass slabs beneath to ensure adequate control of the material. The cements were condensed with an amalgam plugger and filled to excess which was removed with a glass slab until the surface was smooth and level. After the preparation of each sample, it was suspended above a hot water bath at a temperature of 37°C and covered with

a lid to achieve 100% humidity. The samples were then removed from the hot water bath container and tested every 5 minutes with a Vicat needle apparatus (needle mass 300±0.5 g with a 1±0.5 mm flat diameter). The indenter was lowered, and an indent was made on the material surface. The needle was cleaned between each test. Setting time was recorded when the needle did not penetrate surface of the material further.

Compressive strength: (Fig 5 & 6)

For testing the compressive strength, the material was placed into molds of 8 mm high and 4 mm in diameter with a glass slab beneath to ensure a flat surface for condensation. The material was added in increments and condensed with an amalgam plugger to minimize air entrapment. Molds were filled to excess, and the excess material was removed by using a glass slide to ensure level samples. Specimens were suspended on a metal grate above a closed hot water bath (37°C) and covered to provide 100% humidity without submerging the samples to allow for the initial set. After 3 hours, the samples were removed from the molds and examined for voids and irregularities. If voids were detected, the sample was discarded. Each group was tested at 24 hours. The flat surfaces of each sample were sanded smooth with 600 grit sandpaper. The samples were tested by using an Instron Materials Testing M achine until failure. A crosshead speed of 1 mm/sec with a load cell of 5 kN was used. The maximum load required to fracture each specimen was noted, and the compressive strength (C) was recorded in kilograms and converted to MegaPascals (MPa).

Push-out bond strength: (Fig 7)

Ten single-rooted, freshly extracted human teeth were selected for this study. The middle-third of the roots were sectioned perpendicular to the long axis into 1.00 ± 0.05 mm thick slice using a water-cooled diamond blade on a cutting machine. The root slices were drilled with

post drills perpendicular to the root slice to obtain 1.3-mm diameter standardized cavities. The specimens were then randomly divided into two groups. The standardized root slices were filled with MTA and new experimental Calcium Silicate cement. The specimens were wrapped in pieces of wet gauze and were kept for 3 days at 37°C to set. Each group was placed in a separate closed plastic container.

Push-out Test (Fig 8)

The push-out bond strengths were measured using a universal testing machine. The fillings were loaded with a 1-mm diameter cylindrical stainless steel plunger at a speed of 1 mm/min. When dislodgement occurred, the maximum load applied to the fillings was recorded in Newtons. The recorded value was converted to MPa.

Colour stability:

Sample Preparation (Fig 9)

Cylindric samples with a 10-mm diameter and 2-mm high were obtained by allowing them to set in molds prepared similar to ones done for setting time analysis as mentioned in pg no. 24. Specimens were stored at 37°C and 100% humidity during setting for materials to reach their optimal mechanical properties. After each sample was separated from the molds, 1 sample from each group was immersed in the following irrigation solutions for 24 hours: distilled water and 5% NaOCl solution. The samples were dried, and the colors of each were assessed before and after immersion in different solutions.

Spectrophotometric Analysis (Fig 10 & 11)

Spectrophotometer (Vita Easyshade 4.0) was used to measure color under constant laboratory light by the same operator. The device was calibrated before each measurement. Each

measurement was repeated 3 times. The Commission Internationale de l'eclairage (CIE) system was used to calculate the differences in color. The color differences of the samples were calculated. ΔE values that were ≥3.3 were accepted as clinically perceptible discoloration. Images of the samples were taken before and after immersion using a digital camera.

Scanning Electron Microscopy and Energy Dispersive X-ray spectroscopy (Fig 12 & 13)

24 freshly extracted molars were used for this study and were randomly divided into 2 groups. For assessing bioactivity (surface), cavities of 5x5 mm width with a depth of 2 mm were prepared in 12 teeth. And for assessing remineralization (interface), cavities of 5x2 mm width with a depth of 3 mm were prepared in 12 teeth. The teeth were randomly divided into 2 groups and were filled with respective cements i.e., MTA or new experimental Calcium Silicate cement. After filling they were stored in Phosphate Buffered Solution (PBS) at different durations – 24 hours, 7 days and 3 months.

SEM/EDX analysis:

For testing bioactivity, cross sections (3 mm thick) of samples were obtained by cutting them with a water cooled diamond blade perpendicular to the long axis of tooth. For testing remineralization potential, interfacial surface was obtained by cutting the teeth parallel to the long axis of teeth. Bioactivity and remineralization potential were analysed using SEM with EDX at 24 hours, 7 days and at 3 months. The samples were tilted 60° to produce a better topography in the images and micrographs were taken at magnifications of 1000X to 5000X. All elements present in respective cements were detected with the help of EDX.

Fig 1

Fig 2

Fig 3 Fig 4

Fig 5

Fig 6

Fig 7

Fig 8

Fig 9

Fig 10

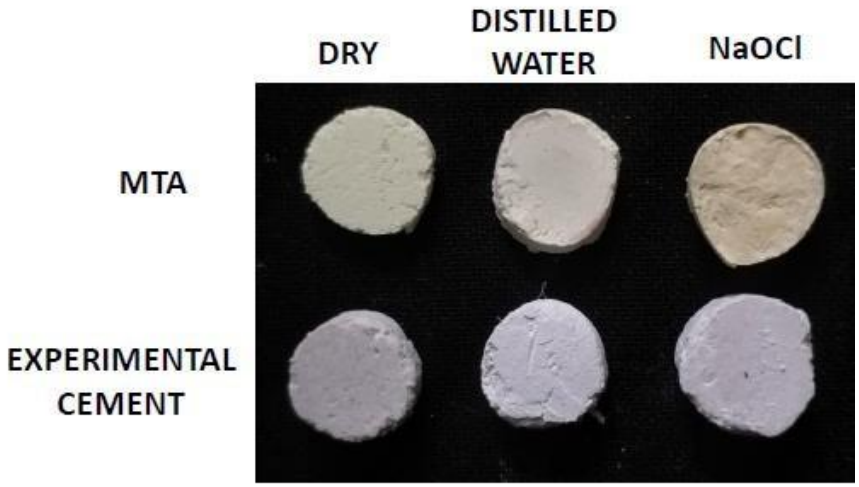


Fig 11

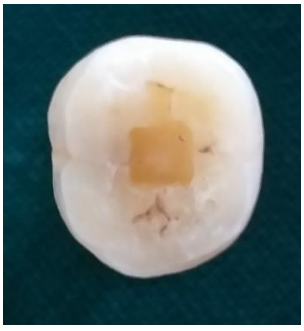


Fig 12

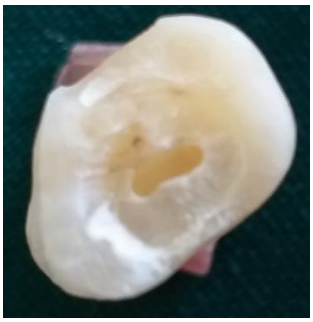


Fig 13

STATISTICAL ANALYSIS

STATISTICAL ANALYSIS

Statistical analysis was performed using Student T test for setting time, compressive strength, push-out bond strength and ANOVA for color stability. And the significance was maintained at 0.05.

Setting time:

Table 1

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Setting time	Equal variances assumed	.103	.757	-6.000	8	.000	-60.000	10.000	-83.060	-36.940
	Equal variances not assumed			-6.000	7.529	.000	-60.000	10.000	-83.313	-36.687

Push-out Bond strength:

Table 2

Independent Samples Test

	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	17.672	.003	-4.854	8	.001	-3.584000	.738389	-5.286728	-1.881272
Equal variances not assumed			-4.854	4.014	.008	-3.584000	.738389	-5.631334	-1.536666

Compressive strength:

Table 3

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Compressive strength	Equal variances assumed	.706	.425	1.768	8	.115	5.000	2.828	-1.522	11.522
	Equal variances not assumed			1.768	7.236	.119	5.000	2.828	-1.644	11.644

Color stability:

Table 4

ANOVA

				Sum of Squares	df	Mean Square	F	Sig.
MTA	Between Groups	(Combined)		82.460	2	41.230	16.064	.004
		Linear Term	Contrast	75.615	1	75.615	29.460	.002
			Deviation	6.845	1	6.845	2.667	.154
	Within Groups			15.400	6	2.567		
	Total			97.860	8			
New experimental Calcium Silicate cement	Between Groups	(Combined)		73.042	2	36.521	6.325	.033
		Linear Term	Contrast	51.042	1	51.042	8.839	.025
			Deviation	22.001	1	22.001	3.810	.099
	Within Groups			34.647	6	5.774		
	Total			107.689	8			

RESULTS

RESULTS

XRD analysis for MTA

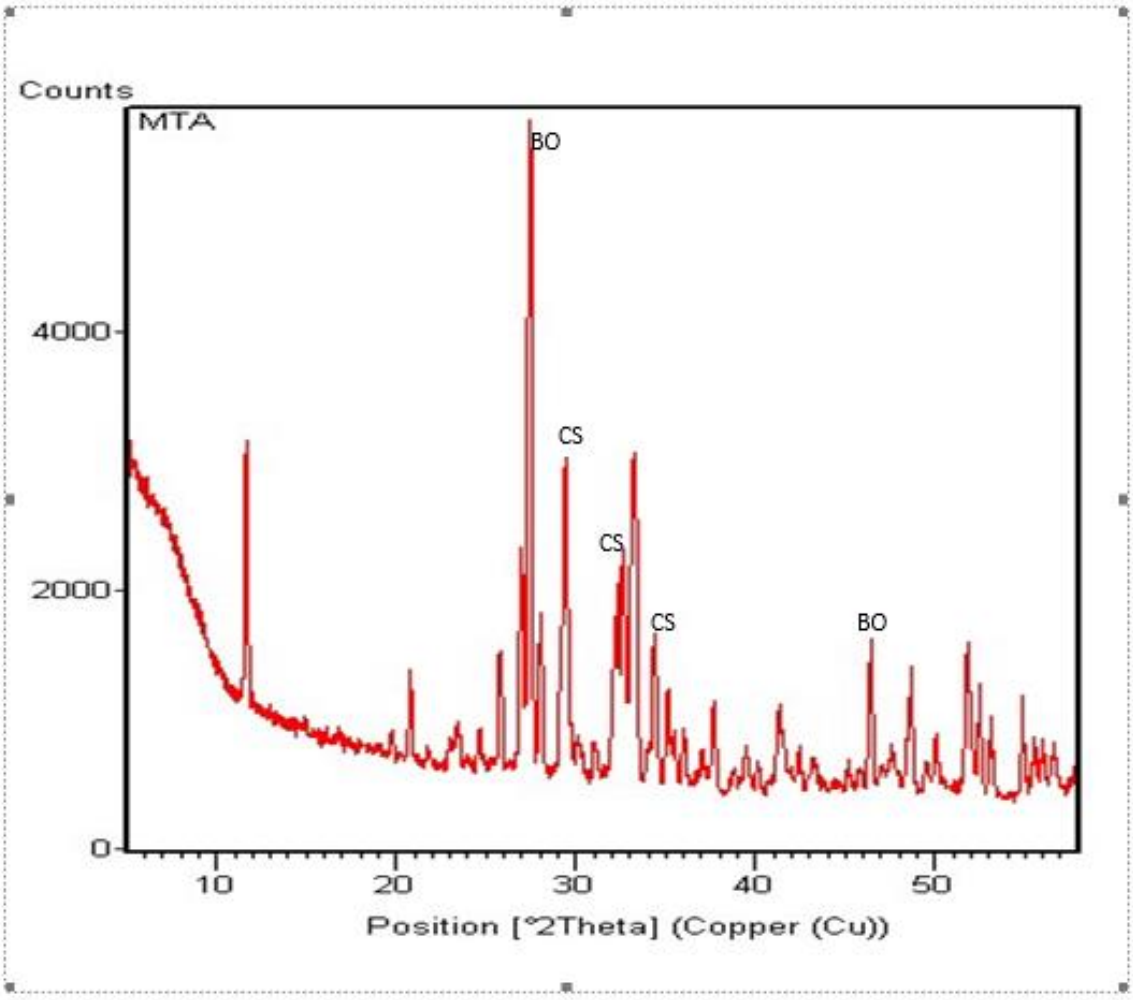


Fig 14

XRD analysis for new experimental Calcium Silicate cement

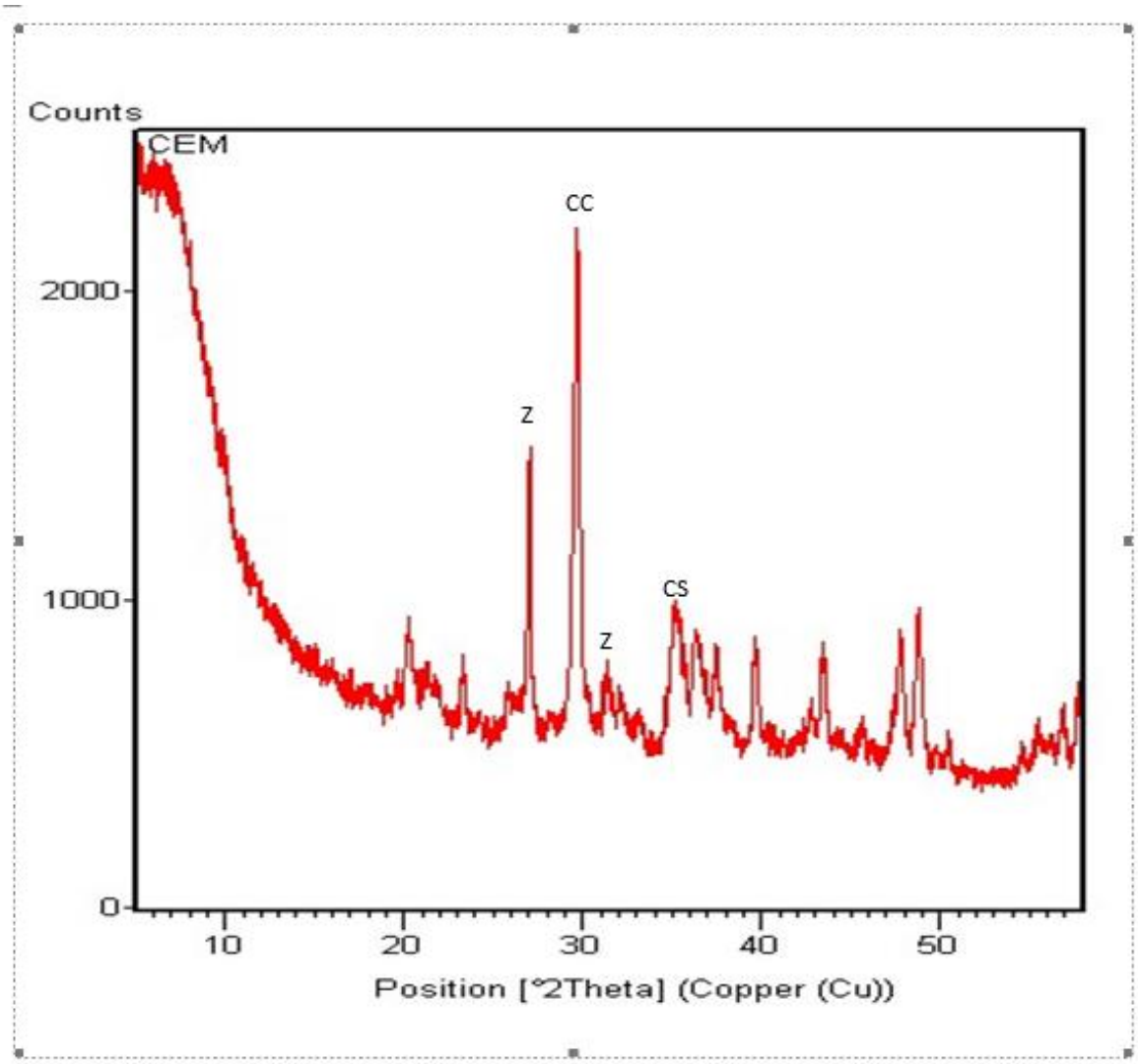


Fig 15

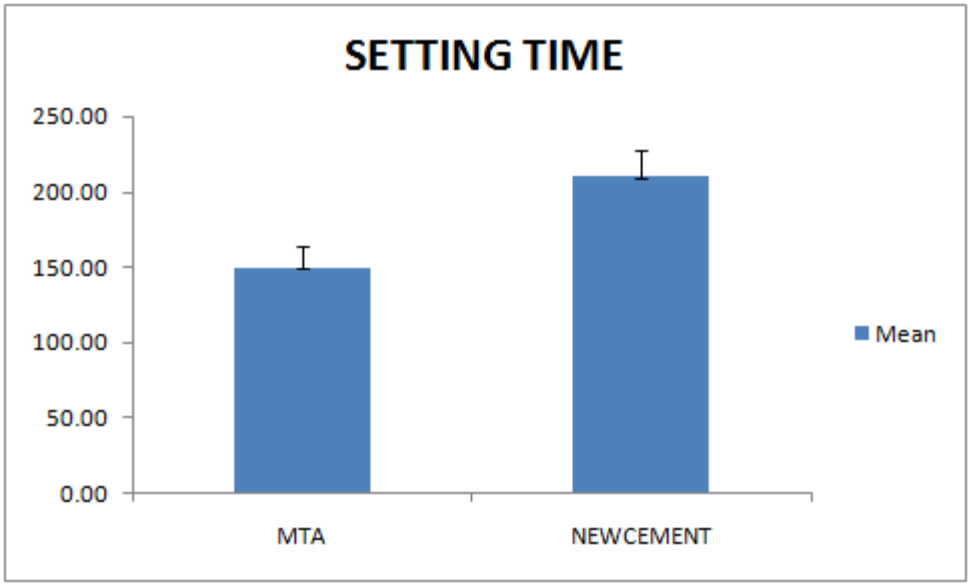

SETTING TIME
250.00
200.00
150.00
100.00
50.00
0.00
MTA
NEWCEMENT
Mean

Fig 16

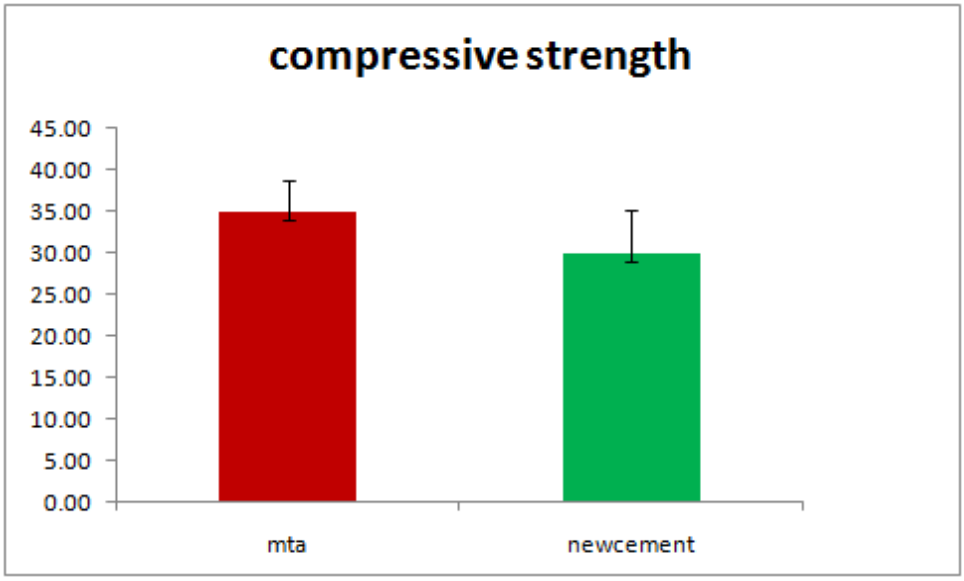

compressive strength
45.00
40.00
35.00
30.00
25.00
20.00
15.00
10.00
5.00
0.00
mta
newcement

Fig 17

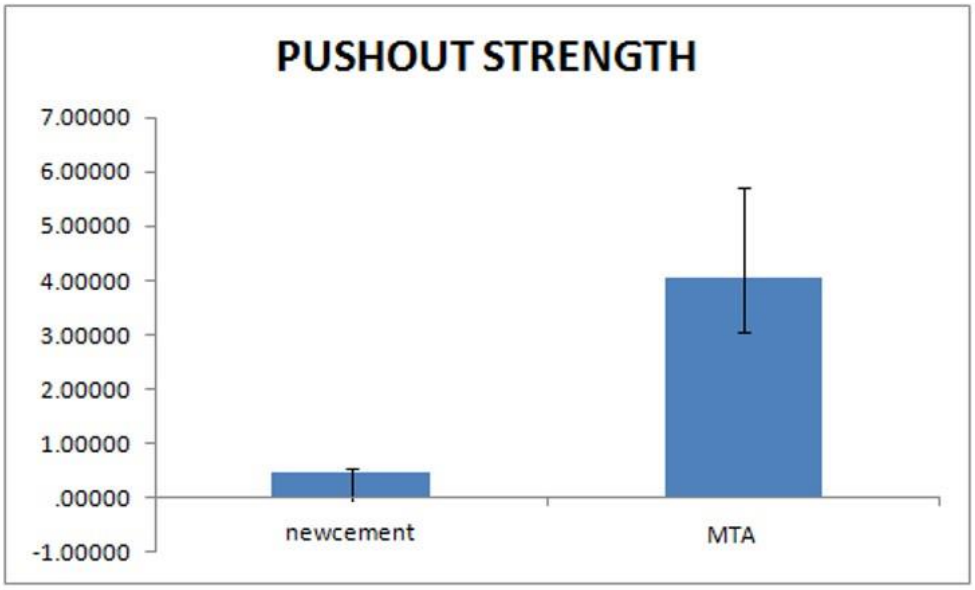

PUSHOUT STRENGTH
7.00000
6.00000
5.00000
4.00000
3.00000
2.00000
1.00000
.00000
-1.00000
newcement
MTA

Fig 8

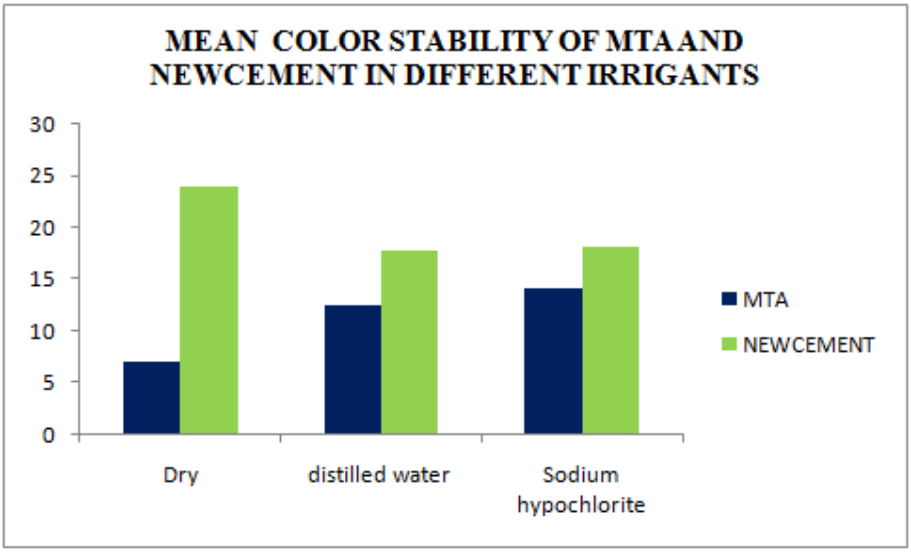

MEAN COLOR STABILITY OF MTA AND NEWCEMENT IN DIFFERENT IRRIGANTS
30
25
20
15
10
5
0
Dry
distilled water
Sodium hypochlorite
MTA
NEWCEMENT

Fig 19

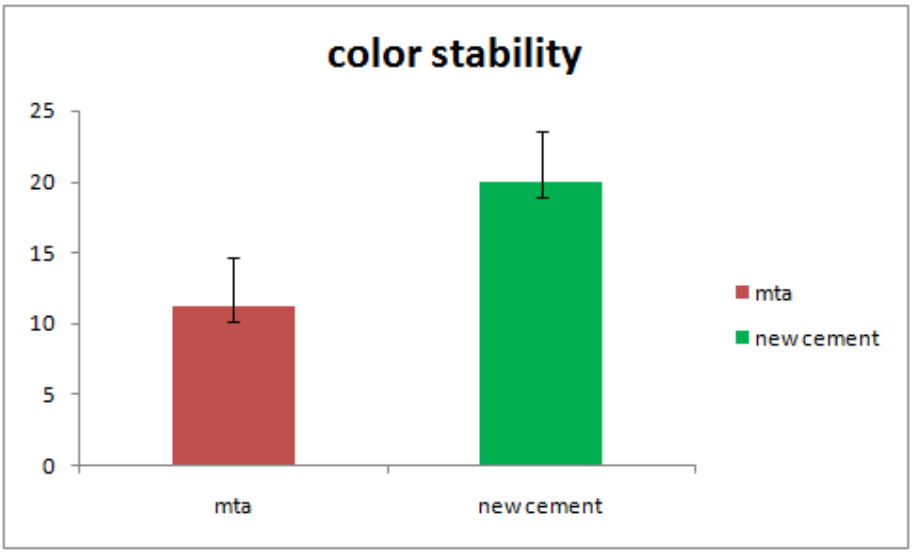
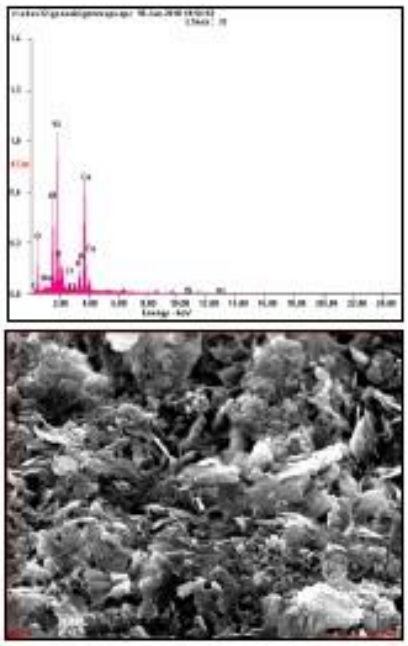

color stability
25
20
15
10
5
0
mta
new cement
mta
new cement

Fig 20

Scanning electron Microscopy and Energy Dispersive X ray Analysis:

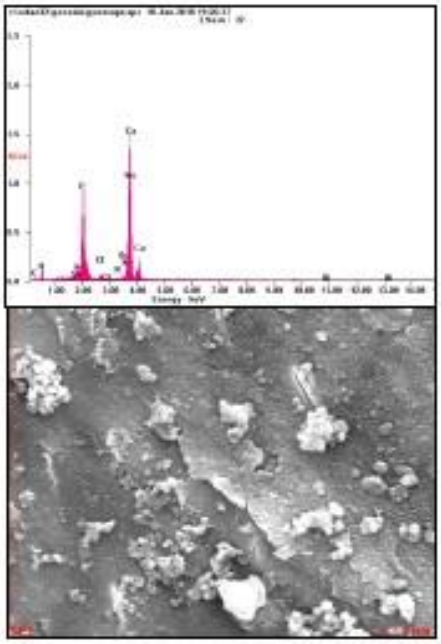
SEM and EDX of Surface of MTA at 24 hours- Ca:P - 1.69



Element	Wt%	At%
CK	13.36	22.56
OK	34.56	43.81
NaK	01.52	01.34
AlK	10.22	07.68
SiK	18.28	13.20
PK	04.93	03.23
ClK	00.82	00.47
KK	02.19	01.14
CaK	12.71	06.43
BiL	01.42	00.14
Matrix	Correction	ZAF
Element	Wt%	At%
PK	31.35	37.14
CaK	68.65	62.86
Matrix	Correction	ZAF

Fig 21

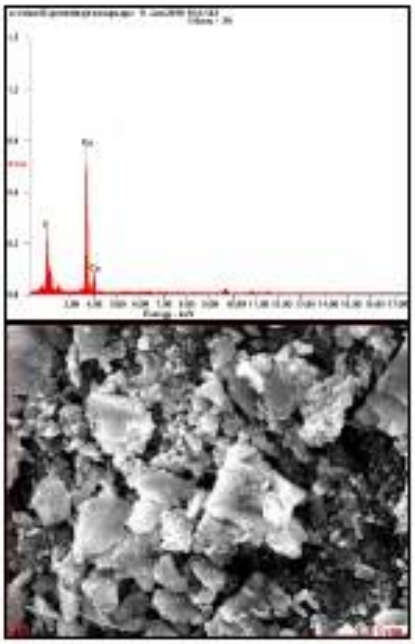
SEM and EDX of interface of MTA at 24 hours - Ca:P - 1.57



Element	Wt%	At%
CK	18.33	33.81
OK	23.50	32.54
SiK	00.06	00.04
SrL	02.54	00.64
PK	18.53	13.26
ClK	00.58	00.36
KK	00.09	00.05
SnL	01.21	00.23
CaK	34.33	18.98
BiL	00.84	00.09
Matrix	Correction	ZAF
Element	Wt%	At%
PK	32.93	38.85
CaK	67.07	61.15
Matrix	Correction	ZAF

Fig 22

SEM and EDX of Surface of new experimental Calcium Silicate cement at 24 hours - Ca:P - 2.11

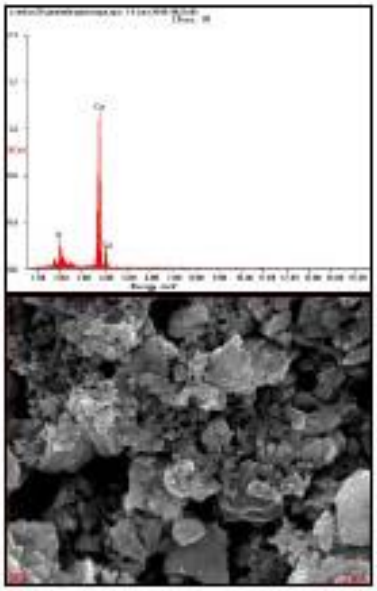


Element	Wt%	At%
CK	10.67	21.37
NK	06.39	10.97
OK	18.82	28.28
SiK	01.38	01.18
PK	14.95	11.61
KK	00.58	00.36
CaK	42.88	25.73
BiL	04.32	00.50
Matrix	Correction	ZAF

Element	Wt%	At%
PK	26.73	32.07
CaK	73.27	67.93
Matrix	Correction	ZAF

Fig 23

SEM and EDX of interface of new experimental Calcium Silicate cement at 24 hours - Ca:P - 3.90

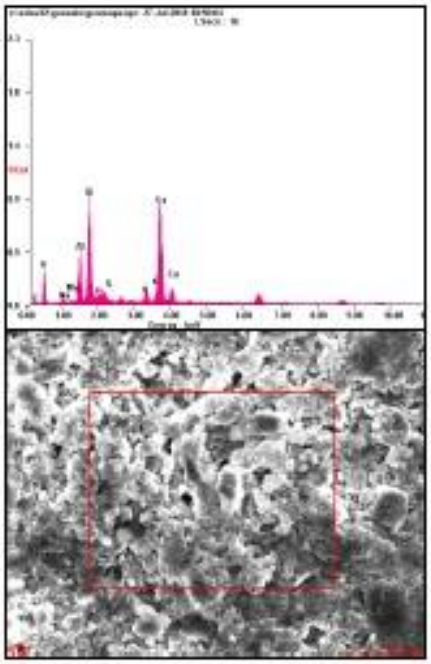


Element	Wt%	At%
CK	12.37	26.52
OK	16.57	26.67
NaK	01.34	01.50
PK	18.20	15.13
TcL	02.13	00.56
CaK	45.32	29.12
BiL	04.09	00.50
Matrix	Correction	ZAF

Element	Wt%	At%
PK	16.53	20.40
CaK	83.47	79.60
Matrix	Correction	ZAF

Fig 24

SEM and EDX of Surface of MTA at 7 days - Ca:P - 2.24

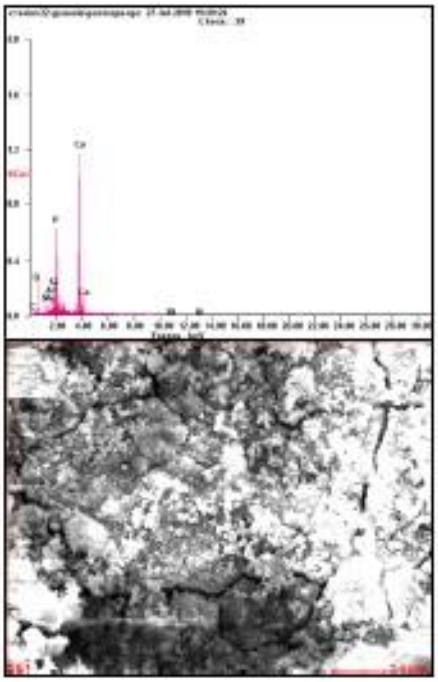


Element	Wt%	At%
CK	16.64	26.81
OK	37.15	44.93
NaK	01.21	01.02
MgK	00.23	00.18
AlK	08.59	06.16
SiK	15.54	10.71
PK	01.39	00.87
SK	00.09	00.05
KK	01.71	00.85
CaK	17.44	08.42
Matrix	Correction	ZAF

Element	Wt%	At%
PK	25.60	30.81
CaK	74.40	69.19
Matrix	Correction	ZAF

Fig 25

SEM and EDX of interface of MTA at 7 days - Ca:P - 1.69

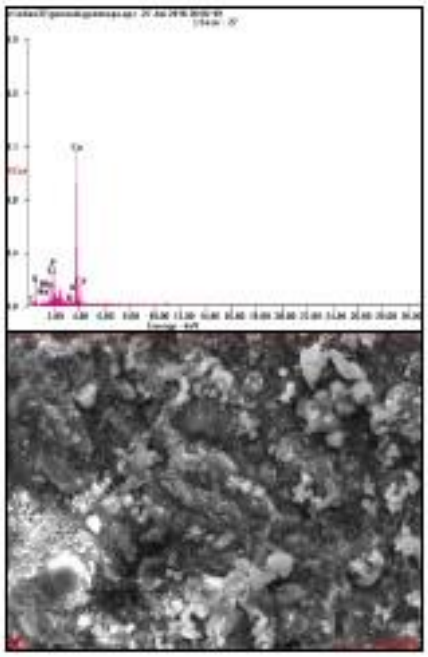


Element	Wt%	At%
CK	14.33	24.28
OK	34.72	44.15
AlK	09.95	07.50
SiK	16.79	12.16
PK	02.38	01.56
SK	00.39	00.25
ClK	00.72	00.41
KK	02.29	01.19
CaK	15.11	07.67
FeK	01.89	00.69
BiL	01.43	00.14
Matrix	Correction	ZAF

Element	Wt%	At%
PK	31.32	37.11
CaK	68.68	62.89
Matrix	Correction	ZAF

Fig 26

SEM and EDX of Surface of new experimental Calcium Silicate cement at 7 days - Ca:P - 2.56

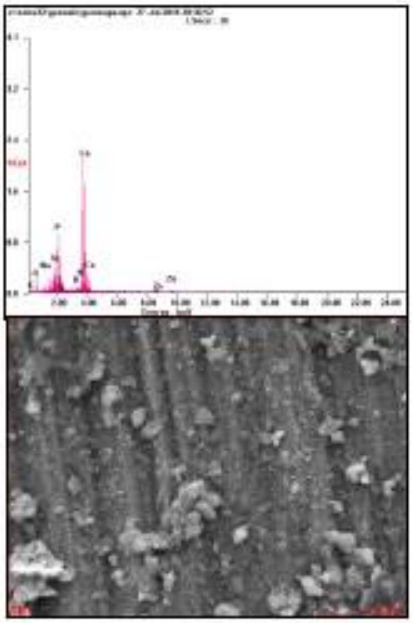


Element	Wt%	At%
CK	11.00	19.27
OK	37.90	49.84
NaK	01.94	01.77
MgK	02.34	02.02
SiK	06.17	04.62
PK	07.22	04.91
KK	00.62	00.34
CaK	32.82	17.23
Matrix	Correction	ZAF

Element	Wt%	At%
PK	23.17	28.08
CaK	76.83	71.92
Matrix	Correction	ZAF

Fig 27

SEM and EDX of interface of new experimental Calcium Silicate cement at 7 days - Ca:P - 1.93

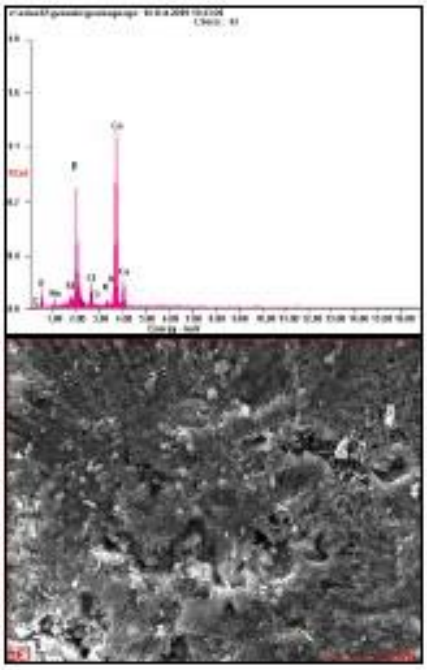


Element	Wt%	At%
CK	16.97	30.29
OK	26.81	35.93
NaK	03.00	02.80
SiK	04.70	03.58
PK	12.80	08.86
SK	00.76	00.51
KK	00.89	00.49
CaK	30.72	16.44
ZnK	03.34	01.10
Matrix	Correction	ZAF

Element	Wt%	At%
PK	28.58	34.12
CaK	71.42	65.88
Matrix	Correction	ZAF

Fig 28

SEM and EDX of Surface of MTA at 3 months - Ca:P - 1.45

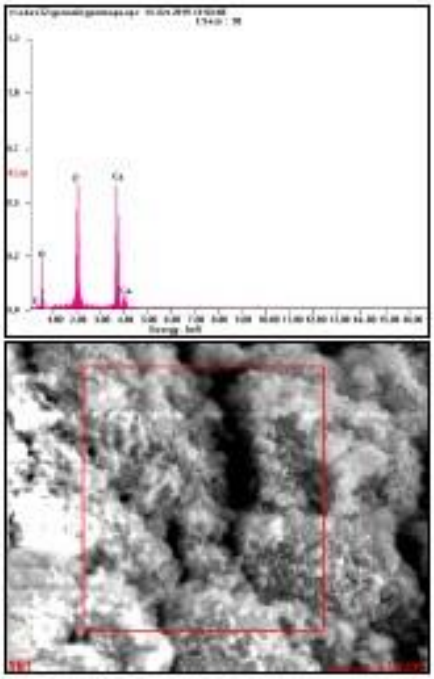


Element	Wt%	At%
CK	13.88	25.48
OK	26.90	37.08
NaK	03.16	03.03
SiK	01.25	00.98
PK	18.18	12.94
SK	00.19	00.13
ClK	03.82	02.38
KK	01.05	00.59
CaK	31.57	17.37
Matrix	Correction	ZAF

Element	Wt%	At%
PK	34.76	40.80
CaK	65.24	59.20
Matrix	Correction	ZAF

Fig 29

SEM and EDX of interface of MTA at 3 months - Ca:P - 1.02

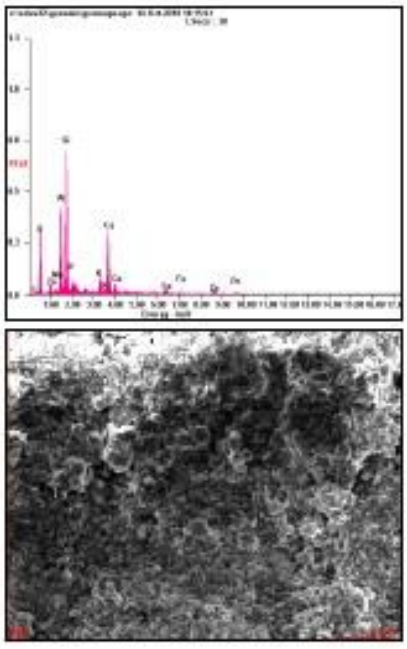


Element	Wt%	At%
CK	24.69	37.10
OK	38.90	43.88
NaK	01.05	00.82
MgK	00.37	00.28
AlK	00.35	00.24
PK	15.83	09.22
CaK	18.79	08.46
Matrix	Correction	ZAF

Element	Wt%	At%
PK	42.88	49.28
CaK	57.12	50.72
Matrix	Correction	ZAF

Fig 30

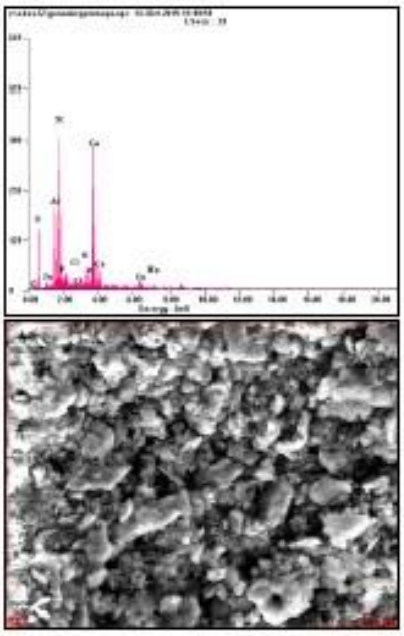
SEM and EDX of Surface of new experimental Calcium Silicate cement at 3 months - Ca:P - 1.74



Element	Wt%	At%
CK	05.71	10.07
OK	40.44	53.50
MgK	00.36	00.31
AlK	13.20	10.36
SiK	21.86	16.48
PK	02.26	01.54
KK	02.40	01.30
CaK	09.20	04.86
FeK	01.85	00.70
ZnK	02.72	00.88
Matrix	Correction	ZAF
Element	Wt%	At%
PK	30.72	36.46
CaK	69.28	63.54
Matrix	Correction	ZAF

Fig 31

SEM and EDX of interface of new experimental Calcium Silicate cement at 3 months - Ca:P - 1.66



Element	Wt%	At%
CK	10.59	18.26
OK	38.21	49.46
ZnL	02.48	00.79
AlK	09.67	07.42
SiK	17.64	13.01
PK	01.53	01.02
ClK	00.57	00.34
KK	01.87	00.99
CaK	15.41	07.96
FeK	02.02	00.75
Matrix	Correction	ZAF
Element	Wt%	At%
PK	31.66	37.48
CaK	68.34	62.52
Matrix	Correction	ZAF

Fig 32

DISCUSSION

DISCUSSION

Calcium silicate cements are being increasingly used in the field of Endodontics in various applications such as pulp capping, root end filling, sealer, etc., due to its bioactivity and remineralization potential enabling a good compatibility of the material with the tissues. Despite its various advantages, several disadvantages do exist such as the long setting time of few materials, high cost, discoloration of tooth and surrounding tissues. To overcome these problems, we have developed a new formulation of Calcium Silicate cement and analyzed a few characteristics of the same.

X-Ray Diffraction analysis:

In our study, MTA showed peaks for calcium silicate (ICDD: 31-0301) at 29.35, 32.461, 34.27° 2θ. And it had Bismuth oxide peaks (ICDD 071- 0465) at 27.3 which was intense. And new experimental Calcium Silicate cement showed peaks for calcium silicate (ICDD: 31-0301) at 34.27° 2θ and a peak for calcium carbonate (ICDD: 05-0586) at 29.35° 2θ. Peaks for the zirconium oxide phase (ICDD: 37-1484) was present at 28.17 and 31.47° 2θ and was intense at 28.17° 2θ

Camilleri et al showed that both BioAggregate and MTA Angelus exhibited peaks for tricalcium silicate (ICDD 031-0301). Triclinic tantalum oxide (ICDD 021-1198) and silicon dioxide (ICDD 082-1576) were present in BioAggregate, while bismuth oxide (ICDD 071- 0465) was present in MTA Angelus. (15) Grech et al in his study showed that the X-ray diffraction plots presented the tricalcium silicate phase (ICDD: 31-0301) for all the tricalcium silicate cement-based materials with peaks at 29.35, 32.461, 34.27° 2θ. The peaks were not very well defined compared to the radiopacifier peaks. BioDentine and TCS- 20-Z cement exhibited peaks for the zirconium

oxide phase (ICDD: 37-1484) at 28.17 and 31.47° 2θ. The zirconium oxide peak in BioDentine was less intense than that of TCS-20-Z. BioDentine also exhibited a peak for calcium carbonate (ICDD: 05-0586) at 29.35° 2θ. Tantalum oxide (ICDD: 33-1390) was present at 23.50, 26.33, 29.81 and 36.33° 2θ in Bioaggregate. Calcium carbonate acts as a nucleation site. Although it is not cementitious as it does not react with water to form reaction by-product; however, it allows the formation of reaction rims around it, thus enhancing the hydration and producing a denser microstructure. (52) Lee et al in his study revealed several new peaks at *2*θ=18°, 28.6°, 34.3°, and 47.1°, related to calcium hydroxide (Portlandite, ICDD 44-1481), were found in the hydrated materials and a peak at 29.4° *2*θ, related to $CaCO_3$ formation (calcite, ICDD 05-0586). (53)

Setting time and Compressive strength:

MTA's longer setting time which has been a major disadvantage was worked on by many researchers to improve it by adding various accelerators in it such as $CaCl_2$, $CaCO_3$, CLG, Na_2HPO_4, etc. Various Calcium silicate cements were thus developed with a decreased setting time. However Dawood et al stated that the long setting time of MTA may be less important, especially when it is used in multiple visits treatments. (54) The setting time of this new experimental Calcium Silicate cement was found to be 210 mins which was significantly greater than that of MTA - 150 mins. Hence we reject the null hypothesis that there will not be significant difference between MTA and new experimental Calcium Silicate cement in terms of setting time.

In BioDentine, decreased setting time was achieved by a combination of different effects. First, particle size greatly influences the setting time, since the higher the specific surface, the shorter the setting. Also, adding calcium chloride to the liquid component accelerates the system. Finally, the decrease of the liquid content in the system decreases the setting time to harden within

9 to 12 minutes. In 2012, **Quick-Set** was introduced with a decreased setting time by refining the Capasio powder and liquid (28)

Flores Ledesma et al used a new experimental Calcium Silicate cement which was prepared using a white Portland cement with 20 wt% of Bi_2O_3. He mixed various concentrations of wollastonite cement and bioactive glass and observed that the setting time was 9.5 min at the maximum wollastonite concentration 30 wt%. However, addition of 10 wt% of bioactive glass had the lowest setting time of about 14.8 minutes. (55) Calcium silicate-based cement (CS) associated to microparticulated (micro) or nanoparticulated (nano) zirconium oxide (ZrO_2) were compared with CS and bismuth oxide (BO) with CS. The addition of radiopacifiers increased the setting time of CS. It is possible that the increase in the setting time caused by ZrO_2 (micro and nano) and BO added to CS may be due to the presence of smaller amount of cement in these mixtures, affecting its hydration and increasing the setting time. (56) Three CSCs incorporated with 0%, 0.5%, 1.0%, 2.0% and 3.0% (w/w) CPP-ACP were investigated. The addition of CPP-ACP to CSCs prolonged their setting times. (54)

Bartoluzzi et al in his study showed that setting time of MTA reduced by 50% when 10% of $CaCl_2$ was added to the powder. Concentration of $CaCl_2$ in our study was 5% which could be attributed to the increased setting time in our Experimental Calcium Silicate cement. And Silva et al showed that material particle size could improve the setting time due to its rapid hydration where he proved that nanoparticulated cement had reduced setting than microparticulated. Since new experimental Calcium Silicate cement had microparticles, setting time could have been increased. Those 2 aspects has to be worked upon to improve the setting time.

The addition of any substance interferes with the hydration reaction of the Portland cement- based cement, leading to delay in matrix formation and increasing the setting time of the

cement. The hydration reaction of MTA is a complex process and any modification of this process might alter the physiochemical and biological properties of the resulting cement. (54)

Compressive strength is an important factor to consider when a filling material is placed in a cavity that bears occlusal pressure. However Torabinejad et al stated that because root-end filling materials do not bear direct pressure, the compressive strength of these materials is not as important as those materials used to repair defects in occlusal surfaces. (6)

Though MTA (35±3.67 MPa) had higher compressive strength compared to the new experimental Calcium Silicate cement (30±5.14 MPa), the difference in compressive strength was not statistically significant. Hence we accept the null hypothesis that there will not be significant difference between MTA and new experimental Calcium Silicate cement in terms of compressive strength. A study using wollastonite and bioactive glass showed reduction in compressive strength. (55) This was also evident in a study done using microparticulated (micro) or nanoparticulated (nano) zirconium oxide (ZrO_2) or bismuth oxide (BO) as additives to CS. (56) Addition of CPP-ACP was also found to reduce the compressive strength. (54)

Push-out Bond strength:

Any cement should remain in place under dislodging forces, such as mechanical loads of occlusion or the condensation of restorative materials over it. Therefore, the push-out bond strength is an important factor for perforation repair materials. (57)

New experimental Calcium Silicate cement showed less push-out bond strength values than MTA which was statistically significant. Hence we reject the null hypothesis that there will not be significant difference between MTA and new experimental Calcium Silicate cement in terms of push-out bond strength. The reason for reduction in push-out bond strength can be

explained by the presence of 5% $CaCl_2$ in new experimental Calcium Silicate cement used in the present study. Almeida et al explains this effect due to a reduction in expansion of the material. Also the immediate contact of MTA+$CaCl_2$ with moisture may alter the powder liquid ratio and reduces the cohesive strength between the cement particles, thus negatively influencing the bond strength to dentine. However, addition of $CaCl_2$ may improve the biomineralization ability of MTA, leading to improvement in the bond strength after a period of time. (58) This favourable response has been found in new experimental Calcium Silicate cement with increased HA formation on its surface and interface at 3 month interval compared to 24 hours and 1 week time period.

Color Stability:

Color changes in dental materials can be measured visually and with specific instruments. The CIE system is an arrangement for international standardization on issues of color and is acknowledged by the ISO. Instrumental measurements of color use the CIE system with colorimeters and spectrophotometers. Visual spectrophotometry is a gold standard method that has been used in dentistry. Spectrophotometric analysis with the Vita Easyshade Compact was applied because of the technique's sensitivity to small changes in color, repeatability, and objectivity. (59)

Sobhamayan et al showed that wMTA exhibited higher discoloration in contact with NaOCl when compared with dry condition, normal saline, and EDTA. Immersion of wMTA in sodium hypochlorite resulted in the formation of a dark brown precipitate that adheres to root canal walls. NaOCl, one of the most commonly used irrigation solutions, has a tendency to crystallize and occlude dentinal tubules and may not be completely removed from root canals. Residual NaOCl in dentinal tubules may make contact with calcium silicate–based materials used for regenerative procedures or to repair resorptions and perforations. It has been speculated that the

discoloration was attributed to the reaction of sodium hypochlorite with bismuth oxide. (60) In our study, set specimens were immersed in irrigation solutions for 24 hours to duplicate prolonged contact of the solution and calcium silicate–based materials. Discoloration was a result of bismuth containing substances contacting with NaOCl. The proposed mechanism behind discoloration was a reduction of NaOCl to sodium chloride. Also Bismuth oxide disassociates into dark color crystals of metallic bismuth and oxygen when exposed to visible and ultraviolet light. (50) Tested materials were allowed to set in molds and immersed in the irrigation solutions after setting. Submersion before setting could theoretically retard the curing process and result in the materials dissolving into the irrigation solutions and could also cause surface porosities that would affect color measurements and possibly provide incorrect results. (59) Similar results were seen with MTA Plus that contains bismuth oxide which exhibited material discoloration in contact with sodium hypochlorite solution. (13) However, newer materials that contained zirconium oxide as a contrasting substance did not have high staining potential. (10) A study by Garcia et al demonstrated that the staining caused by MTA is not related to bismuth oxide, because EndoBinder with and without radiopacifying agent presented a similar performance between them. Because Endobinder is a calcium aluminate cement where its discoloration could be attributed to the presence of aluminium. They also concluded that in spite of the lower concentration of iron oxide, WMTA presented the highest loss of luminosity (darkening) at 360 days than the other groups, demonstrating that the difference in the concentrations of this compound between the two commercial forms of MTA is not the determinant factor in the staining ability of the material. WMTA was introduced into the market as low-iron concentration cement; nevertheless, the cement also promotes tooth darkening due to iron content oxidation, because of tetracalcium aluminoferrite, which indicates that not only the composition of the cements, but also the form of

obtaining and sintering them is related to their ability to stain dental structures. Moreover, Namazikhah et al. reported that the microstructure of Portland-based cements, such as MTA, present pH-dependent porosities, which may uptake staining substances and be responsible for dental structure staining. (14)

In our study MTA exhibited higher discoloration when in contact with distilled water and NaOCl when compared to dry. However there was no statistical difference between distilled water and NaOCl. This might be attributed to the presence of Bismuth oxide in MTA. New experimental Calcium Silicate cement did not undergo discoloration. On the contrary it showed improvement in color when in distilled water and NaOCl than in dry condition. However, there was no statistical difference between them. This could be attributed to the presence of Zirconium oxide which was added as a radiopacifier. Moreover new experimental Calcium Silicate cement which has no aluminium, iron or bismuth in its composition exhibited good color stability when compared to MTA. Hence we reject the null hypothesis that there will not be significant difference between MTA and new experimental Calcium Silicate cement in terms of color stability.

SEM and EDAX:

When Calcium Silicate cements are exposed to Phosphate containing fluids, a solid–liquid interface forms on the mineral particles, and ion dissolution occurs. Ca^{2+} ions rapidly migrate into the mixing solution and Portlandite ($Ca(OH)_2$) forms. The silicates are attacked by OH ion groups in an alkaline environment and a CSH phase forms on mineral particles. CSH has a negative surface charges that may act as nucleation sites for apatite formation. CSH contains an excess of calcium hydroxide/ Portlandite formed by OH^- ions from dissociated water molecules and Ca^{2+}ions from the cement particles. Hence a strong continuous outflux of calcium hydroxide

from CSH occurs during the first few hours after mixing causing a marked rise in the pH and an increase in calcium ion concentration in the surrounding environment. The calcium phosphate apatite deposits form a layer of spherulites filling the superficial porosities. (17)

A biomaterial is deemed bioactive when it elicits a positive specific biological response at the interface, resulting in the formation of a direct bond between host tissue and materials. The outstanding performance of MTA is largely attributable to its bioactivity, which means its capacity to spontaneously produce an interfacial apatite layer when in contact with phosphate-containing physiological fluids (61) HA can release calcium and phosphorus continuously, a process required for bone metabolism. In addition, this phenomenon increases the sealing ability of MTA and promotes the regeneration and remineralization of hard tissues. Interaction between physiologic solutions and various types of MTA (AMTA, MTA Bio) reported that white precipitation forms over the material in the first hour after immersion in PBS, and it completely covers the surface of MTA after 5 days. (62)

The soaking in low-concentration phosphate solutions such as Hank's solution reduced the rate of apatite precipitation and increased the time required for superficial apatite formation. After immersion for 5–24h in the phosphate medium all cements were covered by micro-clusters of nano- and micro-globular crystals composed of calcium and phosphate. (63)

SEM analysis of MTA and new experimental Calcium Silicate cement revealed various surface and interfacial morphologies depending on its immersion time in PBS. At 24 hours of immersion, both showed presence of agglomerates of crystalline particles. However crystal growth increased with time of immersion and at 3 months they had substantial crystal growth which had clusters of spherical particles.

On elemental analysis of cements with EDX, we found that at 24 hours of soaking MTA, its Ca/P ratio was found to be 1.69 and 1.57 at surface and interface respectively which is very close to that of HA. However the Ca/P ratio seemed to decrease at 3 month interval to 1.45 and 1.02 at the surface and interface respectively. Though new experimental Calcium Silicate cement showed higher Ca/P ratio of 2.11 and 3.9 at surface and interface respectively at 24 hours. This is similar to the reports of a study by Gandolfi et al that the Ca/P atomic ratio of the coating of approximate range 1.8-3.0 denotes formation of bone-like carbonate apatite. (34) (64) It seemed to decrease to a Ca/P ratio of 1.74 at surface and 1.66 at interface which is an indication of formation of pure HA at 3 months thus confirming good bioactivity and remineralization potential at 3 months interval. This was in accordance with Zeng H et al where he showed that Ca/P ratio of the surface should be exactly 1.67 to form a pure hydroxyapatite (HA) crystals. Otherwise it will have phases other than HA regardless of the presence of H_2O. (65)

Gandolfi MG et al showed that in MTA Angelus, an irregular CaP coating was present on the surface, and the Ca/P atomic ratio was approx. 2.2. In MTA Plus, the Ca/P atomic ratio at 28 days was 2.62. In ProRoot MTA, The Ca/P atomic ratio was 1.84. (34) However in his another study he showed that after 7 days, a precipitate had formed on MTA Plus that contained compounds of calcium, sodium, magnesium, phosphorous, and chlorine with a Ca/P ratio of 4.45. The carbon peak was no longer observed, indicating the precipitate formed from HBSS completely covering the surface. The atomic percentage of calcium and phosphorous increased for the 28-day sample, the Ca/P ratio was 2.47, which is higher than that of hydroxyapatite. At 28 days in HBSS, Freshly mixed ProRoot MTA surface was coated by globular calcium phosphorous precipitates, with a Ca/P ratio of 1.84; however, bismuth was still detectable. (64)

SUMMARY

SUMMARY

Calcium Silicate cements have successfully emerged as one of the best materials for a wide range of applications from vital pulp therapies to root end filling materials where bioactivity supersedes all other properties. In this study an indigenous new experimental Calcium Silicate cement was formulated to overcome few disadvantages of Mineral Trioxide Aggregate, however to retain its remineralization potential comparing it with Mineral Trioxide Aggregate. Characterization of the cements was done using X-Ray Diffraction analysis, setting time was measured with Vicat needle apparatus, compressive strength and push-out bond strength were assessed in universal testing machine, color stability was tested using a spectrophotometer and bioactivity was studied using Scanning Electron Microscope and Energy Dispersive X-ray spectroscopy. Characterization of cements revealed their constituent elements. Though setting time and push-out bond strength of the new experimental Calcium Silicate cement were unfavorable, it showed a satisfactory compressive strength and good color stability. In fact the new experimental Calcium Silicate cement showed improvement in its color after storage in distilled water and sodium hypochlorite in contrast to Mineral Trioxide Aggregate. There was formation of a bone like carbonate apatite at 24 hours and pure hydroxyapatite at 3 months when the new experimental Calcium Silicate cement was immersed in Phosphate Buffered Solution. Thus with improvement in particle size and cement formulation to accelerate the setting time and increase the push-out bond strength, the new experimental Calcium Silicate cement can prove to be a viable alternative to Mineral Trioxide Aggregate at an affordable cost.

CONCLUSION

CONCLUSION

A new Calcium Silicate cement was experimentally formulated to overcome the disadvantages of long setting time, color instability and cost of MTA. However the setting time was found to be longer for the new experimental Calcium Silicate cement. Also the push-out bond strength was inferior to MTA. Nevertheless the color stability of the new experimental Calcium Silicate cement was superior to MTA even in the presence of sodium hypochlorite. The compressive strength was comparable to MTA. Surface bioactivity and remineralization ability was superior for MTA at 24 hrs and found to decrease at 3 months. But for the new experimental Calcium Silicate cement there was an exponential increase in surface bioactivity and remineralization ability from 24 hrs to 3 months. With suitable modifications to accelerate the setting reaction and increase push-out bond strength, the new experimental Calcium Silicate cement can prove to be a viable alternative to MTA at an affordable cost.

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ANNEXURES

URKUND

Urkund Analysis Result

Analysed Document:	CHARACTERIZATION OF A NEW BIOACTIVE CALCIUM SILICATE CEMENT - Copy.pdf (D62892509)
Submitted:	1/23/2020 6:14:00 PM
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7.DISCUSSION(P) - Copy.docx (D61287109)
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