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EVALUATION OF EFFECT OF INJECTABLE PLATELET RICH FIBRIN ON THE ALKALIZING ACTIVITY AND ION RELEASE OF MINERAL TRIOXIDE AGGREGATE AND BIODENTINE – AN IN VITRO STUDY

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ABSTRACT

Aim: To assess the influence of iPRF on the pH and Ca^{2+} ion release from MTA and Biodentine. **Material and Methods:** Cylindrical samples were prepared of MTA control, MTA mixed with iPRF, BD control and BD mixed with iPRF. After an initial set, the samples were placed in falcon tubes containing 10ml deionized water. This solution was checked for pH followed by Ca^{2+} ion release at intervals of 3h, 24h, 14 days and 28 days. **Results:** pH analysis revealed no significant differences between the tested groups, all groups exhibited alkaline pH. Ca^{2+}ion analysis revealed highest release for BD control followed by BD mixed with IPRF. MTA mixed with iPRF reported higher Ca^{2+} ion release compared to MTA control. **Conclusions:** iPRF did not significantly influence the pH of MTA and Biodentine. Addition of iPRF increased the Ca^{2+} ion release from MTA, while it had no significant difference on the Ca^{2+}ion release of Biodentine at the end of 28 days.

KEYWORDS: iPRF (Injectable -Platelet Rich Fibrin), MTA (Mineral Trioxide Aggregate), BD (Biodentine), ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry)

INTRODUCTION

Vital pulp therapy aims to regenerate the pulp - dentine complex enabling dentine formation. An important function of vital pulp therapy is to stimulate the pulp odontoblasts to lay down reparative dentin and promote remineralization of existing dentin; thus encouraging the dentin–pulp complex and eventually, the carious lesion, to heal (Cohenca et al., 2013). The regeneration of the tissues depends on the interaction between the dental material and the dental tissue. This interaction is influenced by a variety of factors such as composition of the material, the chemistry and concentration of any eluted components or degradation products, and the ability of the tissue to respond to these agents. An understanding of the role of the material on the regeneration of the dental tissue is critical.

A variety of materials have been used by dental clinicians for vital pulp therapy, ranging from calcium hydroxide to resin modified glass ionomer cement, tricalcium phosphate and more recently calcium silicate-based cements e.g. MTA, Biodentine, Calcium enriched mixture, Bioaggregate etc.

While calcium hydroxide remains the gold standard for pulp capping procedures owing to its excellent antimicrobial properties (Accorinte *et al.*, 2008), it presents several limitations such as tunnel defects in dentin barrier, extensive dentin formation obliterating the pulp chamber, high solubility in oral fluids, and lack of adhesion and degradation after acid etching (Cox CF et al., 1998)

In light of the above-mentioned disadvantages related to calcium hydroxide, newer calcium silicate-based cements such as MTA and Biodentine have gained popularity.

Pitt Ford et al., 1996 were the first to evaluate the performance of MTA for pulp capping in monkey's teeth and they observed superior performance of MTA when compared to calcium hydroxide. Since then, several clinical studies and case reports have shown favourable results when MTA was used as a pulp capping agent in permanent teeth. Bogen et al., 2008 observed a 100% success rate when MTA was used as a pulp capping agent in permanent teeth while Farzi et al.,2006 observed a success rate of 93.8% when MTA was used in vital pulp therapy.

MTA is composed primarily of tricalcium, dicalcium silicate and bismuth oxide, which on hydration produce a silicate hydrate gel and calcium hydroxide rendering the material biocompatible. It also presents several advantages over calcium hydroxide such as faster rate of healing and faster calcific barrier formation (Pitt Ford et al., 1996). However, one of the main disadvantages when using MTA is its extended setting time (Torabinejad et al. 1995, Islam et al. 2006), tooth discoloration and difficulty in handling.

Biodentine, another calcium silicate-based product which became commercially available in 2009 and was specifically designed as a "dentine replacement" material. It presents advantages over MTA in terms of its shorter initial setting time of 9 -12 mins which can be attributed to the presence of calcium chloride in the liquid component.

Both MTA and Biodentine undergo similar hydration process. Hydration of bioactive materials leads to the release of a number of ions during the setting stage. Camilleri et al. 2005 demonstrated that on hydration MTA produces Calcium-Silicate-Hydrate(C-S-H) gel and Calcium Hydroxide (CH). The CH is produced from the hydration of tricalcium and dicalcium silicate. The release of calcium from the cements occurs through dissolution of CH and by progressive decalcification of the C-S-H. The high levels of calcium leached out from the cement account for its biocompatibility. The released Ca^{2+} ions also increase alkalinizing activity, have a bactericidal effect, suppress osteoclast activity, and trigger fibroblast formation (Lengheden A et al, 1995). Ca^{2+} can activate Ca-dependent ATPase and react with carbon in the tissues; the subsequent formation of calcium carbonate representing the initiation of remineralization. Ca^{2+} ions are also required for cell migration and differentiation (Mohammadi Z and Dummer PM 2011).

Healing of the tissues is a complex process and it is well known that platelets play an important role in achieving haemostasis and in wound healing. In dentistry platelet concentrates have been utilized for over three decades as a regenerative tool capable of releasing physiological doses of growth factors responsible for inducing tissue regeneration. Platelet concentrates prepared in 2 forms-platelet rich plasma (PRP) and Platelet rich fibrin (PRF).

Whitman et al in 1997, were the first to use platelet-rich plasma in oral surgical procedures and had reported great advantages but its use presented risk as bovine thrombin was used to handle PRP and this may generate anti-bodies to factors V, XI, and thrombin that could cause coagulopathies that may endanger life.

To overcome the disadvantage of PRP, new generation platelet concentrates in the form of PRF was introduced in 2001 by Choukron et al. It is the first source of autogenous blood-derived growth factors harvested without the use of anti-coagulant and is a rich source of growth factors - transforming growth factor β1, platelet-derived growth factor, and insulin-like growth factor, exhibiting speckled characteristic such as cell migration, cell proliferation, cell attachment, and cell differentiation (Harlamb S et al 2016).

A case report by Khetarpal et al, 2013 showed that the combination of PRF as an internal matrix and MTA as a barrier and lead to successful healing and apexification. Bains et al, 2012 reported use of MTA over PRF was effective for the management of pulpal floor perforation and the treatment of an immature tooth with apical periodontitis. Miron et al in 2017 introduced an injectable formulation of PRF (iPRF) which provided an easy to use platelet concentrate in liquid form that can either be utilized alone or easily combined with various available biomaterials. Slower centrifugation speeds of 700 rpm for a short duration of three mins resulted in a higher presence of regenerative cells with higher concentrations of growth factors (Miron et al, 2017). There is limited literature on the use of iPRF in Endodontics.

These platelet concentrates with higher presence of regenerative cells and growth factors, when combined with biomaterials like MTA and Biodentine could positively influence the outcome of vital pulp therapy, enhancing reparative dentine formation and thus pulpal healing. It is important to evaluate the physicochemical and biological properties of such a combination before clinical application.

Hydration of Calcium silicate-based cements results in a colloidal gel that solidifies into a hard structure. Characteristics of the mixture can be influenced by the powder/liquid ratio, method of mixing, pressure used during condensation, humidity of the environment, the type of MTA, the type of storage media, the pH value of the environment, the type of vehicle, the length of time between mixing and evaluation, thickness of the material, and temperature. Several modifications to the

liquid component of MTA have been carried out to enhance its physico-chemical properties. Some of the liquid accelerants tested include 2% Xylocaine with 1:100,000 epinephrine (Sluyk et al., 1998), Calcium Chloride (CC) (Bortuluzzi et al., 2006), sterile water, saline, 2% lidocaine, 3% NaOCl gel, CHX gluconate gel, K-Y Jelly and 3% and 5% CC (Kogan et al., 2008). Various studies have shown that modification of Calcium silicate cements may have an adverse effect on its physical and possibly its bioactive properties. There is a need to develop a liquid component that can improve the properties of these Calcium silicate-based cements and thus enhance its biological effects. There is no literature on the modification of calcium silicate-based cements with platelet concentrates. The hydration of MTA and BD with autogenously sourced iPRF as the liquid component could augment its healing potential.

Research has shown pH and Ca^{2+} release plays important roles in mineralisation and the subsequent hard tissue formation. A high pH contributes to its antibacterial properties and also stimulates the release of ALP and bone morphogenetic protein 2, which participate in the mineralization process (Moghaddame-Jafari S et al., 2005) while Ca^{2+} release enhances the expression of genes associated with odontoblastic differentiation, such as DSP and DMP-1, ALP activity, and mineralization.

Therefore this in vitro study was carried out to assess the influence of iPRF on pH and Ca^{2+} release from MTA and Biodentine.

MATERIALS AND METHODS

- Stainless Steel molds (Bangalore, India)
- MTA ANGELUS (Angelus Indústria de Produtos Odontológicos S/A, Londrina, Brazil)
- Biodentine (Septodont, Saint-Maur-des-Fossés, France)
- 10ml Syringe (Dispovan)
- 2.5ml Syringe (Dispovan)
- Bioline Petroleum Jelly
- Micro Applicator Tips (Oro)
- Glass Slab
- Cement Spatula
- Condensers
- Sterile Cotton
- Plastic Filling instruments
- iPRF tubes (Linkfar Healthcare, St. Franziskus, Germany)
- 15ml falcon Tubes

Cylindrical samples were prepared of MTA control, MTA mixed with iPRF, BD control and BD mixed with iPRF. After an initial set, the samples were placed in falcon tubes containing 10ml deionized water. This solution was checked for pH followed by Ca^{2+} ion release at intervals of 3h, 24h, 14 days and 28 days.

Sample Preparation

IPRF preparation: - Informed consent was obtained from healthy volunteers. Blood was obtained by venipuncture of the antecubital vein. iPRF was prepared as per the protocol described by Miron et al (Miron & Zhang 2018). 4ml human blood was collected in a glass coated vacutainer and immediately centrifuged at 400g for 3 min at room temperature. The upper yellow liquid termed iPRF was aspirated immediately as close as possible to lower red cell layer.

Experimental sample preparation: MTA and Biodentine were manipulated with the iPRF obtained from the above steps. After obtaining desired consistency, cement was loaded into stainless steel molds measuring 8mm diameter and 1.6mm in height and allowed to set. The samples obtained were then transferred to falcon tubes containing 10ml deionized water. This solution was used for pH and Ca^{2+} analysis.

MTA (control) preparation: - MTA was mixed according to manufacturer instructions. Mixing was done using glass slab and metal spatula. The mixed cement was placed into stainless steel molds measuring 8mm diameter and 1.6mm in height. The samples obtained were then transferred to falcon tubes containing 10ml de-ionized water after an initial setting time of ±15 min (Aprillia, Usman and Asrianti, 2018). This solution was used for pH and Ca^{2+} analysis.

Biodentine (control) preparation: - Biodentine was prepared according to manufacturer instructions. Mixing was done using glass slab and metal spatula. The mixed cement was placed into stainless steel molds measuring 8mm diameter and 1.6mm in height. The samples so obtained were then transferred to falcon tubes containing 10ml de-ionized water after an initial setting time of ±12 mins (Aprillia, Usman and Asrianti, 2018) transferred to falcon tubes containing 10ml de-ionized water. This solution was used for pH and Ca^{2+} analysis.

The samples were retrieved prior to pH and calcium ion analysis of the deionized water at the end of each testing interval of 3 hrs, 24hrs, 14 days and 28 days. Each time the retrieved samples were transferred to new falcon tubes containing fresh deionized water. pH of the solution was measured using Digital pH meter (HI5221 Hanna Instruments Cal Check, United States). Ca^{2+}analysis was performed using ICP-OES (Thermofisher ICAP 7400 ICP-OES, Radial N. American).

pH analysis

The digital pH meter (HI5221 HANNA INSTRUMENTS CAL CHECK, UNITED STATES) was calibrated to pH 7 with standard buffer solution before use. The pH was analyzed by placing the refillable calomel electrode into the falcon tube containing 10ml of solution. The electrode was washed with distilled water and wiped dry between readings.

Calcium ion analysis

The same solution used to test the pH was analyzed for Ca^{2+} release at time intervals of 3hrs, 24hrs, 14 days and 28 days. Inductively coupled plasma - optical emission spectrometry (ICP-OES) was used to analyze the Ca^{2+} release.

ICP-OES principle: When plasma energy is given to an analysis sample from outside, the component elements (atoms) are excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the ray's intensity. To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

RESULTS

Statistical analysis- The pH and Ca^{2+} release were presented as mean. Multiple comparison was made using ANOVA and comparison between different intervals made using ANOVA followed by Post Hoc- LSD (Least Significant Difference) test.

pH analysis

All the Groups revealed an alkaline pH at all tested intervals. Group 1 (MTA control) revealed a pH of 11.75 at the end of 3 hrs which increased to 12.25 at the end of 28 days. Group 2 (MTA+ iPRF) resulted in a pH of 11.9 at the end of 3 hrs which increased to 12.23 at the end of 28 days. Group 3 (BD control) revealed a pH of 11.6 at the end of 3 hrs which increased to 12.42 at the end of 28 days. Group 4(BD + iPRF) revealed a pH of 12.13 at the end of 3 hrs which increased to 12.15 at the end of 28 days.

Table 1: pH among the Groups using ONE-WAY ANOVA followed POST HOC LSD TEST at different experimental periods.

Variable	Group	Mean	SD	P Value
MTA control **Group 1**	3 Hours	11.750	0.1291	0.001*
	24 Hours	12.025	0.0957	
	14 Days	12.125	0.1258	
	28 Days	12.250	0.1915	
MTA +iPRF **Group 2**	3 Hours	11.967	0.0816	0.001*
	24 Hours	12.200	0.0894	
	14 Days	12.217	0.0983	
	28 Days	12.233	0.1506	
BD control **Group 3**	3 Hours	11.675	0.25	0.001*
	24 Hours	11.875	0.25	
	14 Days	12.250	0.0577	
	28 Days	12.425	0.05	
BD + iPRF **Group 4**	3 Hours	12.133	0.2338	0.001*
	24 Hours	12.167	0.1633	
	14 Days	12.233	0.0516	
	28 Days	12.150	0.1225	

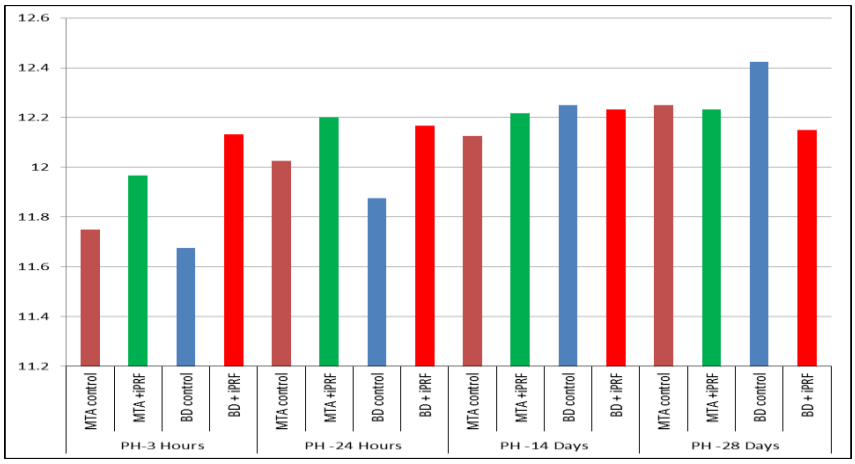

Table 2: Graph representing pH among the Groups at different experimental periods.

Table 3: Multiple Pairwise Comparison of PH at different intervals.

Variable	Comparison between		P Value
PH -3 Hours	MTA control	MTA +iPRF	0.088
		BD control	0.573
		BD + iPRF	0.005*
	MTA +iPRF	BD control	00.026*
		BD + iPRF	0.137
	BD control	BD + iPRF	0.001*
PH -24 Hours	MTA control	MTA +iPRF	0.101
		BD control	0.192
		BD + iPRF	0.178
	MTA +iPRF	BD control	0.005*
		BD + iPRF	0.716
	BD control	BD + iPRF	0.01*
PH -14 Days	MTA control	MTA +iPRF	0.119
		BD control	0.057
		BD + iPRF	0.07
	MTA + iPRF	BD control	0.558
		BD + iPRF	0.742
	BD control	BD + iPRF	0.769
PH -28 Days	MTA control	MTA +iPRF	0.854
		BD control	0.092
		BD + iPRF	0.279
	MTA + iPRF	BD control	0.047*
		BD + iPRF	0.312
	BD control	BD + iPRF	0.007*

Calcium Ion Analysis

Group 1 (MTA control) revealed a Ca^{2+} release of 11.5mmol/mg at 3 hrs which increased to 78.22mmol/mg at the end of 28 days. Group 2 (MTA +iPRF) revealed a release of 6.5mmol/mg at 3 hrs which increased to 98.62 mmol/mg. Group 3 (BD control) revealed a spurt in Ca^{2+} release of 105mmol/mg at 3hrs which increased to 132.3 mmol/mg at the end of 28 days. Ca^{2+} release for Group 4 (BD + iPRF) was 10.9mmol/mg which increased to 118.9 mmol/mg.

Group2 (MTA+IPRF) displayed a higher release of Ca^{2+} at the end of 28 days when compared to Group1(MTA control) while Group3 (BD Control) and Group4(BD+iPRF) revealed no significant difference after 28 days.

Table 4: Ca^{2+} (mg/L) release among the Groups using ONE-WAY ANOVA followed POST HOC LSD TEST at different experimental periods.

Variable	Group	Mean	SD	P Value
3 Hours	MTA control	11.5	12.2714	0.001*
	MTA +iPRF	6.500	1.4805	
	BD control	105.025	3.0902	
	BD + iPRF	10.967	10.4871	
24 Hours	MTA control	6.7675	1.24901	0.003*
	MTA +iPRF	14.7683	3.67000	
	BD control	19.9175	5.66097	
	BD + iPRF	15.4783	4.42664	
14 Days	MTA control	90.5275	27.43250	0.001*
	MTA +iPRF	111.2617	18.03089	
	BD control	161.2250	12.14434	
	BD + iPRF	117.5667	9.57970	
28 Days	MTA control	78.22	10.30384	0.001*
	MTA +iPRF	98.6250	10.64644	
	BD control	132.3950	10.26244	
	BD + iPRF	118.9733	14.21494	

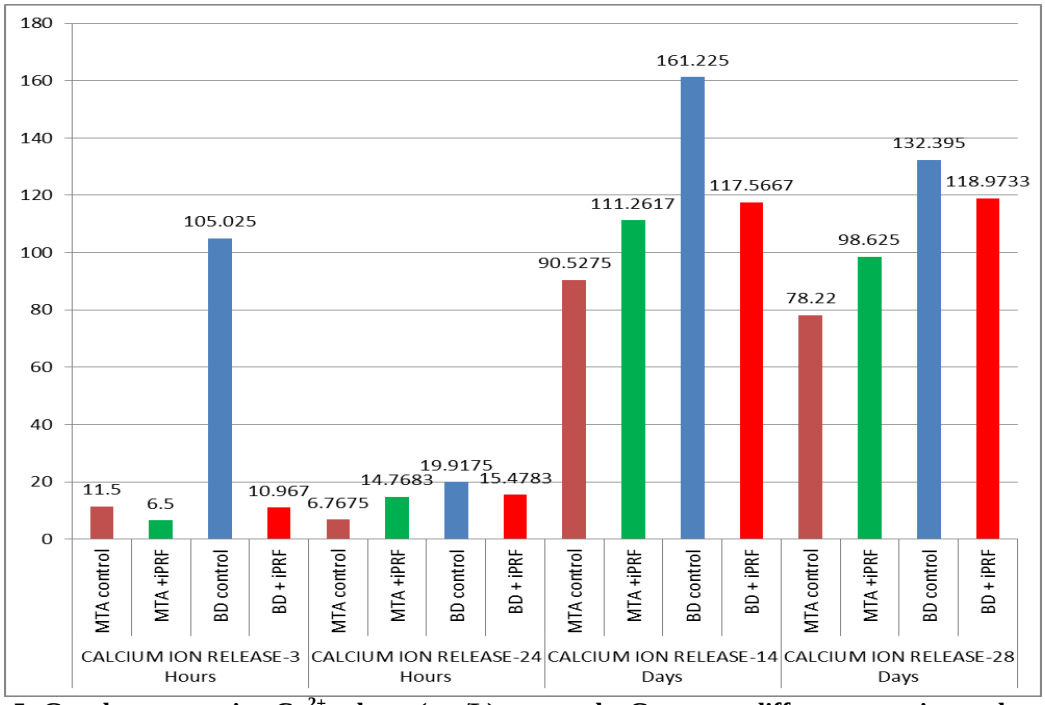

Table 5: Graph representing Ca^{2+} release (mg/L) among the Groups at different experimental periods.

Table 6: Multiple Pairwise Comparison of Ca^{2+} release.

Variable	Comparison between		P Value
3 Hours	MTA control	MTA +iPRF	0.351
		BD control	0.001*
		BD + iPRF	0.920
	MTA +iPRF	BD control	0.000*
		BD + iPRF	0.352
	BD control	BD + iPRF	0.001*
24 Hours	MTA control	MTA +iPRF	0.008*
		BD control	0.001*
		BD + iPRF	0.004*
	MTA +iPRF	BD control	0.068
		BD + iPRF	0.767
	BD control	BD + iPRF	0.111
14 Days	MTA control	MTA +iPRF	0.082
		BD control	0.001*
		BD + iPRF	0.028*
	MTA +iPRF	BD control	0.001*
		BD + iPRF	0.537
	MTA control	BD + iPRF	0.001*
28 Days	MTA control	MTA +iPRF	.016*
		BD control	0.001*
		BD + iPRF	0.001*
	MTA + iPRF	BD control	0.001*
		BD + iPRF	0.009*
	BD control	BD + iPRF	0.096

DISCUSSION

Vital pulp therapy is performed to maintain and preserve the pulp in a healthy state. The procedures vary from Indirect pulp capping (IPC), Direct pulp capping (DPC) and Pulpotomy. These procedures offer good alternatives to root canal therapy for teeth with immature or mature apices, offering a more conservative approach. IPC is a procedure in which carious dentin closest to the pulp is preserved to avoid pulpal exposure and is covered with a biocompatible material (AAPD guidelines on pulp therapy in primary and young permanent teeth., 2008). Recent studies show high survival rate for permanent teeth after performing IPC, without adverse clinical symptoms or pathologic signs on radiographs (Maltz et al., 2007). Direct pulp capping (DPC) is the treatment of a mechanical or traumatic vital pulp exposure by sealing

the pulpal wound with a biomaterial placed directly on exposed pulp to facilitate formation of reparative dentin and maintenance of pulp vitality (American Association of Endodontists guideline, 2003). Ultimately, the goal of treating the exposed pulp with an appropriate pulp capping material is to promote the dentinogenic potential of the pulpal cells (Shroder et al., 1985).

Vital pulp therapy has a high success rate if the following conditions are met: (Cohenca, Paranjpe and Berg, 2013)
(1) The pulp is not inflamed
(2) Hemorrhage is properly controlled
(3) A nontoxic capping material is applied
(4) The capping material and restoration seal out bacteria

Researchers have investigated various materials to aid pulpal healing, including Calcium Hydroxide, Zinc Oxide, Calcium Phosphate, Zinc Phosphate, Zinc Polycarboxylate, Calcium Tetracycline Chelate, Resin-Modified Glass Ionomer Cements, Tricalcium Phosphates, Hydrophilic Resins, Emdogain, Bioglass and more recently calcium silicate-based cements.

An ideal pulp capping material should adhere to tooth substrate, maintain a sufficient seal, be insoluble in tissue fluid, dimensionally stable, non-resorbable, nontoxic, noncarcinogenic, nongenotoxic, radiopaque, and exhibit biocompatibility and bioactivity (Camilleri J et al., 2006).

Calcium hydroxide has been considered the gold standard for pulp capping; however, recent research has shown that : (Cohenca, Paranjpe and Berg, 2013)

- it adheres poorly to dentin
- there is porosity of the dentinal bridge formed with tunnel defects which can act as pathways for microleakage (Cox et al. 1985)
- the cement has tendency to dissolve over time (Schuurs et al., 2000) and is unable to provide a long-term seal against microleakage

Another pulp capping material recently developed is mineral trioxide aggregate (MTA). MTA has demonstrated significantly greater frequency of dentin bridge formation, thicker and less porous dentin, better sealing ability, low solubility, radiopacity and less pulp inflammation and dentinogenic and osteogenic potential compared with calcium hydroxide (Cohenca, Paranjpe and Berg, 2013).

Paranjpe et al, 2010 observed that MTA, when placed in direct contact with the human dental pulp cells, differentiated them into odontoblast-like cells. Holland et al., 2001 reported that at the cellular level, MTA has also been shown to induce the recruitment and proliferation of undifferentiated cells to form a dentinal bridge, while reducing inflammation compared with calcium hydroxide. Nair et al., 2004 demonstrated decreased inflammation when MTA was used compared with calcium hydroxide. Cavalcanti BN., 2004 reported that MTA induced the secretion of angiogenic factors, such as vascular endothelial growth factor, which play an important role in healing. Animal studies by Silva et al., 2008 and Rezende et al., 2008 reported that MTA down-regulated inflammatory cytokines, such as interferon-gamma, CCL5 and IL-1a, 38 and suppressed the proliferation of some microorganisms and inhibited the production of certain TH1 and TH2 cytokines.

Despite its many advantages it also presents several disadvantages such as difficulty in handling and application, long setting time of 175 minutes (Torabinajad M et al., 1995) and coronal discoloration (Accorinte ML et al., 2008) (Parirokh M, Torabinejad M., 2010). Long setting times are an obstacle to using MTA as a pulp capping material since MTA needs to be layered with other materials while it is still fresh (Bogen et al., 2008).

Biodentine (BD) another calcium silicate-based cement has the advantage of a shorter setting time of twelve minutes. The powder consists of tricalcium silicate, dicalcium silicate, calcium carbonate, calcium chloride, and zirconium oxide as a radiopacifier. Invitro and in vivo studies have reported that the mechanism of action of BD is similar to that of MTA. In addition, BD has a positive effect on pulpal cells and helps them form reparative dentin (Gandolfi et al., 2013).

The regenerative potential of platelets was explored as early as the 70's. It was observed that they contain growth factors that are responsible for increase in collagen production, cell mitosis, blood vessels growth, recruitment of other cells that migrate to the site of injury, and cell differentiation induction, among others. Platelet concentrates contain a suspension of growth factors, which act as bioactive surgical additives that can be applied locally to induce wound healing. Many variations of platelet concentrates have been researched on, of which Platelet rich plasma (PRP) and Platelet rich fibrin (PRF) have gained much attention.

PRP is a first-generation platelet concentrate. It has widely been used in orthopaedic surgery, aesthetic medicine and maxillofacial surgery. It provides an ideal growth factor (GF) delivery system to the site of injury. However, it also presents risk as it utilizes bovine thrombin in its preparation, which may generate anti-bodies to factors V, XI, and thrombin and could cause coagulopathies that may endanger life (Marx RE et al., 2004).

PRF, a second-generation platelet concentrate was first used in 2001 by Choukroun et al and is currently considered as a new generation of platelet concentrate. It consists of an autologous leukocyte-platelet rich fibrin matrix, composed of a tetra molecular structure, with cytokines, platelets, cytokines, and stem cells within it (Dohan DM et al., 2006). It is easier to prepare and as it

does not require any chemical manipulation of the blood, it is strictly an autologous preparation.

Injectable form of PRF (iPRF) (Miron et al.,2017) was recently introduced to provide clinicians an easy to use platelet concentrate in liquid form which can be either utilized alone or combined easily with various biomaterials. It requires slower and shorter centrifugation speeds and has shown a higher presence of regenerative cells with higher concentrations of growth factors compared to other formulations of PRF. The rationale behind obtaining injectable PRF is that it contains all components of PRF, including platelets in an uncoagulated form.

Previous in- vitro and in vivo studies have demonstrated that pulpal wound healing by the deposition of mineralized apatite depends on pH and Ca^{2+} release (Holland et al.,2002) (Okabe et al.,2006). Ca^{2+} as well as hydroxyl ions released from the capping materials, regulate the event leading to tertiary dentinogenesis. For the biological effects of calcium hydroxide, the release of bioactive molecules, either through direct stimulation of cells or by solubilization of dentin extracellular matrix, is vital. Calcium silicate cement together with microcrystals deposited on its surface provide a biologically active substrate for the adsorption of biomolecules and adhesion of odontoblasts (Sangwan et al.,2013).

Kang et al.,2013 performed a study comparing the biocompatibility and the release of calcium ions of MTA with hydration accelerators. 4 Groups were assessed: the control Group wherein the samples were prepared by mixing white ProRoot MTA with distilled water. In Groups 2–4, MTA was mixed with 10 wt% Calcium chloride, 32wt% Citric acid, and 43.4 wt% calcium lactate gluconate solution, respectively. The Ca^{2+} ion analysis revealed highest release in Group4(MTA + 43.4 wt% CLG) recording a value of 419.2 ppm followed by Group 2 (MTA + 10 wt% CaCl2) at a value of 331.1ppm and lowest in MTA control sample. Prasad et al.,2015 assessed the physicochemical properties of MTA with various additives such as Ca^{2+}chloride, calcium formate and disodium hydrogen orthophosphate and observed alkaline pH in all tested Groups. MTA+ Calcium chloride recorded lower pH when compared to control while both MTA + calcium formate and MTA + disodium hydrogen orthophosphate recorded higher pH compared to control. A study by Andrade et al., 2014 assessed the pH and calcium release MTA associated with different substances: 1% sodium hypochlorite gel, 2% chlorhexidine gel, K-Y gel, distilled water associated with 10% propylene glycol (CCPG), saline solution, and distilled water alone. It was reported that the K-Y group presented the lowest pH and calcium release values in the 3-hour period. The CCPG group showed a high pH level, which might be due to the presence of calcium chloride in this mixture. For the 24-hour period, the 2% CHX gel group presented the highest pH level, followed by distilled water (control), saline solution and 1% NaOCl gel groups. CHX (2% gel). Calcium ion analysis revealed that the distilled water group presented higher values at the 3, 72 and 168-hour periods. A study by Woo et al., 2015 assessed the combination of MTA with PRF on the odontoblastic differentiation of HDPCs and compared with MTA or PRF alone. An MTA extract was prepared (MTA) and combined with PRF extract (PRFe). Biological effects on HDPCs were assessed. Results revealed cell viability was increased in presence of PRFe while no inhibition in cell growth was seen in HDPCs with MTA combined with PRFe. MTA and PRFe (1.25%) combination enhanced mineralization compared with MTA alone and slightly increased mineralization compared with PRF (1.25%) alone. MTA with PRFe enhanced the expression of BMP2/4 and phosphorylation of Smad1/5/8 compared with the treatment of PRFe (1.25%) alone, whereas treatment with MTA showed no change. The study revealed that the combination of MTA and PRFe promotes the differentiation of HDPCs into odontoblast- like cells through the activation of the BMP/SMAD signaling pathway and the effect of combination is synergic compared with the effect of PRF or MTA alone.

None of the biomaterials available today have been able to satisfy all the requirements of an ideal agent for VPT. Various dental materials and their modifications have been developed to obtain maximum benefits from them and generate the most beneficial tissue response so as to improve the clinical outcome. In this study an attempt was made to modify MTA and BD with liquid platelet concentrate (iPRF) and to assess its effect on the pH and Ca^{2+}release from MTA and BD at different time intervals.

Role of alkaline pH

The pulp capping materials like MTA and BD that are based on tricalcium silicate release calcium hydroxide as a by-product of hydration. (Camilleri J et al.,2008) (Camilleri J et al.,2001). The release of hydroxyl ions creates an alkaline environment which adversely affects the bacterial survival and proliferation. This is important because the antibacterial properties are primarily required at the dentine/restoration interface where residual bacteria could further increase the risk of re-infection (M. G. Gandolfi et al., 2012). Hydroxyl ions stimulate the release of ALP and bone morphogenetic protein 2, which participate in the mineralization process (Moghaddame-Jafari S et al.,2005). To assess the amount of eluted hydroxyl ions from the pulp capping materials, pH values of the aqueous medium are measured.

In the present study all the groups recorded alkaline pH at all intervals. The mean pH recorded in Group 1(MTA control) was 11.7 at 3 hrs which increased to 12.02 at 24 hrs. The pH further increased to 12.1 at 14 days and 12.2 at the end of 28 days. When iPRF was added to MTA (Group 2) pH of 11.9 was recorded at 3 hrs which

increased to 12.20 at 24 hrs, 12.21 at 14 days and 12.23 at the end of 28 days.

When pH of Group 1(MTA control) and Group 2(MTA+ iPRF) were compared, both Groups exhibited a gradual rise in pH over the 28 days period and the difference in values obtained at 3hrs,24hrs,14 days and 28 days were not statistically significant ($p<0.05$). The results revealed that iPRF maintained the alkalising ability of MTA over the 28-day period.

Group3 (BD control) recorded an initial pH of 11.6 at 3 hrs which increased to 11.8 at 24 hours,12.25 at 14 days and 12.4 at the end of 28 days. When iPRF was added to BD (Group 4) an initial pH of 12.13 was recorded at 3 hrs, which increased to 12.16 at 24 hrs and 12.23 at 14 days but dropped to 12.1 at 28 days. On comparison of Group 3 and Group 4, Group 4 recorded higher mean pH at 3hrs and 24 hrs and the results were statistically significant($p<0.05$). pH measurement at 14 days revealed no statistically significant difference in Group 3 and Group 4. At the end of 28 days, Group 3(BD control) recorded a higher mean pH when compared to Group 4 (BD+IPRF) and the results were statistically significant ($p<0.05$). It was observed that addition of iPRF to BD lowered the pH of the media compared to BD control at the end of 28 days.

When Group1 (MTA control) and Group 3 (BD control) were compared, no significant differences in pH were observed at all intervals of 3 hrs,24 hrs,14 days and 28 days. Likewise, when pH values of Group2(iPRF and MTA) and Group 4 (BD and iPRF) were compared no significant differences were observed at all tested intervals. The incorporation of iPRF to BD revealed higher pH at 3hrs and 24 hrs and a drop in pH at the end of 28 days.

On comparison of Group 2 (MTA and iPRF) and Group 4 (BD and iPRF), no statistically significant difference was observed in pH over the 28-day period.

Role of Calcium ions

Ca^{2+} release is a key factor for successful regeneration because the mineralization of cells (osteoblasts, cementoblasts, pulp cells, and odontoblasts) are influenced by Ca^{2+} (Gandolfi et al.,2013). Ca^{2+} specifically modulate osteopontin and bone morphogenetic protein-2 levels during pulp calcification (Rashid et al.,2003). In addition, eluted Ca^{2+} increase the proliferation of HDPCs in a dose-dependent manner (Takita et al.,2006), and Ca^{2+} release enhances the activity of pyrophosphatase, which helps to maintain dentine mineralization and the formation of dentin bridge (Estrela.,2003). Hunter et al 2017 reported that Ca^{2+} released from silicate based material may play an important role in the repair process because they can pass through the cell membranes by depolarization or activation of membrane-bound calcium channels. Ca^{2+} stimulate the expression of bone-associated proteins mediated by calcium channels (Jung et al 2010), and large quantities of Ca^{2+} could activate ATP, which play a significant role in the mineralization process.

When the Ca^{2+} release of MTA (Group 1) was assessed, it was observed that there was an initial fall from 11.5mmol/ml at 3hrs to 6.7mmol/mol at the end of 24 hrs. The Ca^{2+} release peaked to 90mmol/ml on the 14th day and further decreased to 78.2 mmol/ml at the end of 28 days. When iPRF was added an initial rise in Ca^{2+} was observed from 6.5mmol/mg at 3 hrs to 14.7 mmol/ml at 24 hrs which peaked to 111.2mmol/ml at 14 days and fell to 98.6 mmol/ml on the 28th day. Addition of iPRF to MTA improved the release of Ca^{2+} over the 28-day period which was significant ($p<0.05$).

The Ca^{2+} release of BD control (Group 3) at 3 hrs was 105.02 mmol/ml which dropped to 19.9mmol/ml at 24 hrs. It rose to 161.22 mmol/ml at 14 days and 132.3 mmol/ml at the end of 28 days. When iPRF was added (Group 4), the values were 10.9mmol/ml at the end of 3 hrs, 15.4mmol/ml at 24hrs, which rose to 117.5mmol/ml at 14 days and stabilized to 118.9mmol/ml at the end of 28 days. Addition of iPRF had an influence on the Ca^{2+}release pattern of BD which recorded lower values at all time periods as compared to BD alone.

When the mean Ca^{2+} release of Group 1 (MTA control) and Group 3 (BD controls) were compared, BD control performed better ($p<0.001$) at all time periods. When the Ca^{2+} release of Group 2 (MTA+ iPRF) and Group 4(BD +iPRF) were compared, no significant difference was observed at 3hrs,24hrs and 14 days. At the end of 28 days Group 4(BD+iPRF) recorded a higher Ca^{2+} release compared to Group 2(MTA+ iPRF) ($p<.009$) which was statistically significant.

Ca^{2+} analysis revealed higher release from the BD Groups (Group3,4). Highest release was recorded for BD control (Group 3). This could be attributed to the presence of calcium chloride added as an accelerator in the liquid (Bortullozi et al.,2006) and due to its higher solubility when compared to MTA (Nareerat Thanavibul et al., 2019). The higher release of Ca^{2+} from Group 4(BD +iPRF) compared to Groups 1(MTA control) and Group 2 (MTA +iPRF) can also be attributed to its fine particle size and to the presence of calcium carbonate in the powder component of BD that serves as a source of free calcium ions that are present in solution as soon as the powder is mixed to the liquid (Camilleri J.,2013).

The addition of iPRF did not significantly affect the pH of MTA and BD at all tested periods. Addition of iPRF to MTA resulted in higher Ca^{2+} release during the 28-day period compared to MTA control. While the addition of iPRF to BD resulted in no significant difference in Ca^{2+} release over the 28-day period.

The pH analysis and Ca^{2+} release pattern of the present study is in accordance to a study by Nareerat Thanavibul

et al 2019 which assessed the effects of blood contamination on the apatite formation, pH and ion release of three calcium silicate-based materials. ProRoot MTA (WMTA), Biodentine and TotalFill BC RRM putty (TRRM) were used with and without blood contamination. Stainless steel moulds measuring 5 mm in diameter,2 mm in height were used. The materials were prepared in accordance to manufacturer's instructions and loaded into the moulds contaminated with blood. The pH analysis revealed that each material exhibited an alkaline pH (11.03-12.14) at each time point. No significant differences were found between conditions of the same material at any time point. Biodentine and TRRM displayed a significantly higher pH compared with WMTA at each time point ($P<0.05$). There was no significant difference in Ca^{2+} release between conditions of the same material at each time point. However, the Biodentine/non-blood and Biodentine/blood Groups showed a significantly higher Ca^{2+} release compared with the WMTA/non-blood and WMTA/blood Groups at each time point ($P<0.05$).

In a study by Wang et al.,2021, a mineralizing film consisting of hydroxypropylmethylcellulose (HPMC) and polyaspartic acid-stabilized amorphous calcium phosphate (PAsp-ACP) nanoparticles was prepared for biomimetic remineralization. The study revealed that the preferred Ca^{2+}release pattern was an initial spurt followed by a sustained release. This is consistent with previous studies by Li Z et al.,2021, Vergaro V et al.,2011, Yang Y et al., 2017 and Li J et al.,2021.

The present study revealed an initial spurt in Ca^{2+} release for Group 3(BD control) followed by a sustained release during the 28day period. Group 1(MTA control), Group2 (MTA +IPRF) and Group 4 (BD +IPRF) revealed a gradual rise in Ca^{2+} in support of sustained release pattern till the 14^{th} day. A drop-in release was seen at the 28^{th} day for Group 1, Group 2 and Group 3, while Group 4 (BD +iPRF) recorded a rise from 117 to 118 at the 28^{th} day but the difference was not statistically significant ($p=0.8$).

Experimental models and techniques vary in different studies and this could influence the pH and Ca^{2+} release. Material composition could also impact the pH and subsequent ion release such as ProRoot MTA, which contains sulfur, and its setting mechanism differs slightly from that of MTA Angelus. Previous studies by Shekhar et al.,2019 have used polyethylene tubes to assess pH and Ca^{2+} release, the present study used molds similar in size used by Gandolfi et al.,2014 (8 mm diameter x 1.6 mm). The medium for storage of samples may also affect the readings. The present study used deionized water as the storage media since use of saliva or oral tissue fluid may mask the release of Ca^{2+} as saliva itself is a source of Ca^{2+}/phosphate and may lead to calcium phosphate nucleation. The variation in methodology can also result in disparity in results as previous study by Shekhar et al.,2019 used a microelectrode to measure pH directly from the material mass. Present study used a pH meter that not only allows pH measurement at periods longer than the setting time but also actually measuring the materials ability of alkalization. The vehicles employed could also affect the dissociation capacity of the cements, Estrela et al reported that distilled water allows the fastest and most significant dissociation. Various methods are used to evaluate Ca^{2+} release such as titration method, molecular fluorescent probes, UV spectroscopy, Raman spectroscopy, Atomic absorption spectrophotometry and Inductively Coupled Plasma–Optical Emission Spectrometry (ICP-OES). The present study utilized ICP-OES for Ca^{2+} analysis owing to its high sensitivity for inorganic elements. It also provides rapid and multi-element analysis of the solutions.

Further physico mechanical, antibacterial, biocompatibility studies need to be performed before validating the above experimental cements for clinical use in vital pulp therapy procedures.

CONCLUSION

Within the limitations of the present study, it can be concluded that**:**

1. Addition of iPRF to MTA and BD did not significantly affect the pH over a 28-day period.
2. The Ca^{2+} release from all Groups peaked on the 14 day.
3. Addition of iPRF to MTA resulted in higher Ca^{2+} release compared to MTA control over a 28-day period.
4. BD Groups exhibited higher Ca^{2+} release over the 28-day period.
5. Addition of iPRF to BD did not significantly affect its Ca^{2+} release compared to BD control over the 28-day period.

REFERENCES

1. Accorinte, M. de L. R. *et al.* (2008) 'Evaluation of Mineral Trioxide Aggregate and Calcium Hydroxide Cement as Pulp-capping Agents in Human Teeth', *Journal of Endodontics*, 34(1): pp. 1–6. doi: 10.1016/j.joen.2007.09.012.
2. Amini Ghazvini, S. *et al.* (2009) 'Ion release and pH of a new endodontic cement, MTA and Portland cement.', *Iranian endodontic journal*, 4(2): pp. 74–8. doi: 10.22037/iej.v4i2.1354.
3. Aprillia, I., Usman, M. and Asrianti, D. (2018) 'Comparison of calcium ion release from MTA-Angelus® and Biodentine®', *Journal of PHysics: Conference Series*, 1073(5). doi: 10.1088/1742-6596/1073/5/052008.
4. Cohenca, N., Paranjpe, A. and Berg, J. (2013) 'Vital Pulp Therapy', *Dental Clinics of North America*, 57(1): pp. 59–73. doi: 10.1016/j.cden.2012.09.004.
5. Gala, M. *et al.* (2020) 'Solubility, pH change, and calcium ion release of low solubility endodontic mineral trioxide aggregate', *Bulletin of the National Research Centre*, 44(1): pp. 1–5. doi: 10.1186/s42269-020-00303-1.

6. Gandolfi, M. G. *et al.* (2011) 'Development of the foremost light-curable calcium-silicate MTA cement as root-end in oral surgery. Chemical-pHysical properties, bioactivity and biological behavior', *Dental Materials*, 27(7): pp. 134–157. doi: 10.1016/j.dental.2011.03.011.
7. Gandolfi, M. G. *et al.* (2014) 'Ion release, porosity, solubility, and bioactivity of MTA plus tricalcium silicate', *Journal of Endodontics*, 40(10): pp. 1632–1637. doi: 10.1016/j.joen.2014.03.025.
8. Gandolfi, M. G. *et al.* (2015) 'Calcium silicate and calcium hydroxide materials for pulp capping: Biointeractivity, porosity, solubility and bioactivity of current formulations', *Journal of Applied Biomaterials and Functional Materials*, 13(1): pp. 1–18. doi: 10.5301/jabfm.5000201.
9. Hwang, Y. C. *et al.* (2011) 'Chemical constitution, pHysical properties, and biocompatibility of experimentally manufactured portland cement', *Journal of Endodontics*, 37(1): pp. 58–62. doi: 10.1016/j.joen.2010.09.004.
10. Jamali Zavare, F. *et al.* (2020) 'Chemical modification of MTA and CEM cement to decrease setting time and improve bioactivity properties by adding alkaline salts', *Journal of Dental Research, Dental Clinics, Dental Prospects*, 14(1): pp. 1–11. doi: 10.34172/joddd.2020.001.
11. Massi, S. *et al.* (2011) 'PH, calcium ion release, and setting time of an experimental mineral trioxide aggregate-based root canal sealer', *Journal of Endodontics*, 37(6): pp. 844–846. doi: 10.1016/j.joen.2011.02.033.
12. Natale, L. C. *et al.* (2015) 'Ion release and mechanical properties of calcium silicate and calcium hydroxide materials used for pulp capping', *International Endodontic Journal*, 48(1): pp. 89–94. doi: 10.1111/iej.12281.
13. Sáez, M. D. M. *et al.* (2017) 'Evaluation of pH and calcium ion diffusion from calcium hydroxide pastes and MTA.', *Acta odontologica latinoamericana : AOL*, 30(1): pp. 26–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28688183.
14. Shashank, S. *et al.* (2019) 'Comparative pH and calcium ion release in newer calcium silicate-based root canal sealers', *Endodontology*, 31(1): p. 29. doi: 10.4103/endo.endo_53_18.
15. Thanavibul, N., Panichuttra, A. and Ratisoontorn, C. (2019) 'Effects of Blood Contamination on Apatite Formation, pH and Ion Release of Three Calcium Silicate-based Materials', 69(3): pp. 324–333. doi: 10.14456/jdat.2019.40.
16. Tomás-Catalá, C. J. *et al.* (2017) 'Comparative analysis of the biological effects of the endodontic bioactive cements MTA-Angelus, MTA Repair HP and NeoMTA Plus on human dental pulp stem cells', *International Endodontic Journal*, 50(September), pp. e63–e72. doi: 10.1111/iej.12859.
17. Vitti, R. P. *et al.* (2013) 'Chemical-pHysical properties of experimental root canal sealers based on butyl ethylene glycol disalicylate and MTA', *Dental Materials*, 29(12): pp. 1287–1294. doi: 10.1016/j.dental.2013.10.002.
18. Woo, S. M. *et al.* (2016) 'Combination of Mineral Trioxide Aggregate and Platelet-rich Fibrin Promotes the Odontoblastic Differentiation and Mineralization of Human Dental Pulp Cells via BMP/Smad Signaling Pathway', *Journal of Endodontics*, 42(1): pp. 82–88. doi: 10.1016/j.joen.2015.06.019.
19. Yang Y, Jin P, Zhang X, Ravichandran N, Ying H, Yu C, et al. New epigallocatechin gallate (EGCG) nanocomplexes co-assembled with 3-mercapto-1-hexanol and β-lactoglobulin for improvement of antitumor activity. J Biomed Nanotechnol, 2017; 13: 805–14.