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YEDİTEPE UNIVERSITY
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DEPARTMENT OF PEDIATRIC DENTISTRY

EVALUATION of pH and CALCIUM RELEASE of NOVEL CALCIUM-BASED MATERIALS USED in VITAL PULP THERAPIES

DOCTOR OF PHILOSOPHY THESIS

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Director of Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

BURCU SANEM TURAN

10.04.2021

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ABBREVIATIONS AND SYMBOLS

%	: Percentage
wt%	: Weight Percentage
°C	: Celsius Degree
°F	: Fahrenheit Degree
AAS	: Atomic Absorption Spectroscopy
BMP	: Bone Morphogenetic Protein
Ca^{2+}	: Calcium Ion
CEM	: Calcium-Enriched Mixture
CH	: Calcium Hydroxide
DFSCs	: Dental Follicle Stem Cells
DPC	: Direct Pulp Capping
DPSCs	: Dental Pulp Stem Cells
EDTA	: Ethylene Diamine Tetra Acetic Acid
FGF	: Fibroblast Growth Factor
H^+	: Hydrogen Ion
ICP	: Inductively Coupled Plasma Spectrometry
IGF	: Insulin-Like Growth Factor
IL-1β	: Interleukin-1 Beta
IPC	: Indirect Pulp Capping
L/P	: Liquid to Powder Ratio
nm	: Nanometer
mA	: Microampere
mg/L	: Milligrams per Liter

mm : Millimeter

mL : Milliliter

MTA : Mineral Trioxide Aggregate

mV : Microvolt

mW/cm² : Milliwatts per Square Centimeter

NaOCl : Sodium Hypochlorite

OH^- : Hydroxyl Ion

PDLSCs : Periodontal Ligament Stem Cells

pH : Power of Hydrogen

ppm :Parts per Million

SCAPs : Stem Cells From the Apical Papilla

SHEDs : Stem Cells From Human Exfoliated Deciduous Teeth

TGF : Transforming Growth Factor

VPT : Vital Pulp Therapy

ABSTRACT

TURAN B.S. 2021. Evaluation of pH and Calcium Release of Novel Calcium-Based Materials Used in Vital Pulp Therapies. Yeditepe University Institute of Health Sciences, Doctorate Thesis, Istanbul.

The aim of this study is to evaluate and compare the pH values and the calcium release of various pulp capping materials, *in vitro.* Calcium hydroxide-based materials; Dycal, Ultra-Blend plus and calcium silicate-based materials TheraCal LC, MTA Angelus, Biodentine and were prepared and placed into molds (diameter: 8.0±0.1 mm; thickness: 1.6±0.1 mm) (n=10). Set samples were immersed in the 10 mL of deionized water. On Day 1, 3, 7,14 and, 28 the storage media were collected for measurement of pH and calcium release and refreshed. The measurements of pH were carried out with a pH-meter (pH 2100 series; pH/mV/Ion/°C/°F Meter with RS232 and Recorder output; OAKTON Instruments, Vernon Hills, IL USA) and the amount of calcium release was determined using an Atomic Absorption Spectrophotometer (Zeenit 700 P, Analytik Jena AG, Germany). All the material groups released both hydroxyl and calcium ions over 28 days which decreased gradually. Exceptionally, the Dycal group showed a tendency to increase regarding calcium ion release. The mean pH was found significantly highest in the MTA Angelus and Biodentine groups in short term (Day 1 and 3) ($p<0.005$); and Dycal and Biodentine groups in long term (Day 28) ($p<0.05$). Biodentine group showed significantly the highest amount of calcium ion release among all the material groups in Day 1 ($p=0.0001$); also highest in Day 28 except Dycal group ($p<0.05$). On Day 28 the least amount of calcium ion release was measured in the Ultra-Blend plus group among all material groups ($p=0.0001$). There were no statistically significant differences between the mean amount of calcium ions in MTA Angelus, Dycal, and Ultra-Blend plus groups in Day 1; however, on Day 28, the Dycal material group showed significantly higher calcium ion release than the other material groups except Biodentine ($p<0.05$). As conclusion; both calcium silicate and calcium hydroxide-based materials tested in this *in vitro* study were found successful in the release of products required for hard tissue formation. Especially Biodentine group released significantly more calcium and hydroxyl ions in short term. In long term; Biodentine still showed statistically significantly the highest pH and calcium release among all material groups; except the Dycal material group.

Keywords: TheraCal, MTA Angelus**,** Biodentine, Calcium release, pH

ÖZET

TURAN B.S. 2021. Vital Pulpa Tedavilerinde Kullanılan Güncel Kalsiyum Bazlı Materyallerin pH ve Kalsiyum Salımının Değerlendirilmesi. Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi, İstanbul.

Bu çalışmanın amacı; pulpa kaplamasında kullanılan kalsiyum esaslı materyallerin, pH ve kalsiyum salımı değerlerini *in vitro* koşullarda karşılaştırmalı olarak değerlendirmektir. Kalsiyum hidroksit esaslı materyaller; Dycal, Ultra-Blend plus ve kalsiyum silikat esaslı materyaller TheraCal LC, MTA Angelus ve Biodentine hazırlanıp, kalıplara yerleştirilmiş (çap: 8,0 ± 0,1 mm; kalınlık: 1,6 ± 0,1 mm) (n = 10) ve10 mL deiyonize suya konmuştur. 1, 3, 7, 14 ve 28. günde solüsyonlar, pH ve kalsiyum salımı ölçümü için toplanmış ve yenilenmiştir. pH ölçümleri pH-metre (pH 2100 serisi; pH / mV / Ion / ° C / ° F Metre ile RS232 ve kaydedici çıkışı; OAKTON Instruments, Vernon Hills, IL ABD) ve kalsiyum salımı, Atomik Absorbsiyon Spektrofotometresi (Zeenit 700 P, Analytik Jena AG, Almanya) ile yapılmıştır. Tüm materyaller 28 gün içinde, hidroksil ve kalsiyum iyonları salmış; ayrıca Dycal grubunda gözlenen kalsiyum salımındaki artış eğilimi dışında, bu salım zamanla azalmıştır. 1 ve 3. Gün, en yüksek pH, MTA Angelus ve Biodentine gruplarında gözlenirken ($p<0,005$); 28. Günde ise en yüksek değerler Dycal ve Biodentine gruplarında ölçülmüştür ($p<0,05$). Biodentine grubunun 1. Günde en yüksek kalsiyum salım değerlerine sahip olduğu ($p=0,0001$); 28. Günde de Dycal grubu dışındaki tüm materyal gruplarından anlamlı düzeyde yüksek olduğu görülmüştür ($p<0,05$). 28. Günde, tüm gruplar arasında en düşük miktarda kalsiyum iyon salımı, Ultra-Blend plus materyal grubunda ölçülmüştür ($p=0,0001$). 1. Günde MTA Angelus, Dycal ve Ultra-Blend plus grupları arasında anlamlı fark yokken; 28. Günde Dycal materyal grubu Biodentin dışındaki tüm materyal gruplarından daha yüksek miktarda kalsiyum iyon salımı göstermiştir ($p<0,05$). Sonuç olarak; bu in vitro çalışmada değerlendirilen kalsiyum hidroksit ve kalsiyum silikat esaslı mataryellerin, sert doku oluşumu için gerekli iyonların salınımında başarılı olduğu gözlenmiştir. Özellikle Biodentine materyal grubunun, kısa dönemde diğer gruplardan daha fazla hidroksil ve kalsiyum iyonu serbestlediği, uzun dönemde ise Dycal materyal grubu dışındaki tüm gruplardan istatistiksel olarak anlamlı düzeyde daha yüksek pH ve kalsiyum iyon salım değerlerine sahip olduğu görülmüştür.

Anahtar Kelimeler: MTA Angelus**,** Biodentine; TheraCal, kalsiyum salınımı, pH

1. INTRODUCTION AND PURPOSE

Dental caries is the consequence of shifting of the oral biofilm to a cariogenic one dominated by acidogenic and aciduric bacteria. By the reduction of detrimental bacteria and re-establishment of the balance in the biofilm, remineralization could be stimulated, thus caries progression and lesions could be arrested. After sealing of the cavity, the number of bacteria within the carious dentin decreases over time (1,2). Studies and improvements in biomedical research help the development of modern treatment approaches. Novel therapies aim to regenerate the dentin-pulp complex. It is important to evaluate dentinogenesis regulation and dental tissue repair at cellular and molecular levels to implement clinical usage of new materials (3).

The aim of vital pulp therapy (VPT) is to stimulate hard tissue formation, protect the tooth from irreversible pulpal damage, preserve the function and vitality of the tooth and the surrounding tissues which is affected by caries, traumatic injuries and other causes (3,4). Especially in young permanent teeth with immature roots, the continuity of apexogenesis process is very important. Apexogenesis is a histological term used to describe the continued physiological development and formation of the root's apex (5). It is also crucial to protect the primary teeth until the normal exfoliation period to preserve the mesiodistal width and the arch length (6).

When repair is necessary in the dentin-pulp complex, the pulp forms a dentin-like matrix which is called tertiary dentin. Reperative and reactionary dentin occur at the dentin-pulp border due to type of the injury. With the help of the VPT, reversible pulpal damage occurred by caries, traumas, or restorations can be healed (7). VPT involves indirect pulp capping (IPC), direct pulp capping (DPC), and pulpotomy procedures (3).

Calcium hydroxide (CH) is the gold standard agent used for vital pulp treatment for many years; however due to some of its drawbacks, new materials were introduced in the recent years. These novel materials are called as calcium silicate-based materials. These materials are classified as hydraulic cements such as mineral trioxide aggregate (MTA); modified MTA's and resin-modified MTA cements. Release of calcium and hydroxyl ions from these materials after placement like CH stimulates the regeneration of pulp tissue (8). These new materials' clinical success when compared to CH; such as

lower solubility, lower water sorbtion, higher calcium release, higher pH, thicker dentin bridge formation, faster pulp healing; have been shown in different studies (9–12).

Calcium hydroxide and calcium silicate containing materials are used in vital pulp treatments because of the antibacterial environment and hard tissue forming properties created by calcium and hydroxyl ions released from these materials over time. While the basic environment formed by hydroxyl ions provides antibacterial properties, the released calcium ions stimulate stem cells and provides proliferation, migration, odontogenic marker expression and mineralization. In this way, tissue vitality is maintained (13,14).

In recent years, it became very important to avoid unnecessary root canal treatments and ensure the continuity of vitality, so many novel materials containing calcium hydroxide and calcium silicate in content are being produced and many studies investigating the various properties of these materials are carried out. Different materials have been evaluated in terms of calcium release and pH using different variables.

The aim of this study was to comparatively evaluate the calcium release and pH values of novel calcium silicate materials and a conventional setting calcium hydroxide material, *in vitro.*

2. GENERAL INFORMATION

2.1. Dentin Responses to Different Stimuli

2.1.1. Dentinogenesis

Dentinogenesis is the formation of dentin structure by odontoblasts which is derived from ecto-mesenchymal cells on the outer wall of dental pulp. In the post-mitotic state the odontoblasts secrete primary dentin. After primary dentinogenesis, the bulk of the dentin is formed. Secondary dentin is secreted after root formation is finished and secretion continues throughout life. This secretion is slower than primary dentin formation. Therefore pulp chamber size is reduced with the age. The original primer odontoblasts, responsible for primary dentinogenesis, remain in resting situation for life. If the dentin-pulp complex is injured, they are reactivated. Tertiary dentin formation is the example of this survival (15).

2.1.2.Tertiary Dentin

Pulp reacts in two ways to insults, either necrosis or a defensive reaction may occur. For defensive reaction three main mechanisms are involved. First increase of intratubular calcification, second, peritubular dentin deposition, and third, the formation of tertiary dentin. All of these mechanisms aim to reduce dentin permeability, and protect the pulp. Tertiary dentin formation is the most significant, because besides reducing permeability, it also increases the distance between the pulp and the irritation source (16).

Tertiary dentin is formed in response to injury at specific sites. Examples of these injuries are dental caries, traumatic or mechanical injuries. Due to the severity of these injuries; different types of tertiary dentin are formed differently or they form at the same time. These tertiary dentin types are defined as reactionary dentin and reparative dentin (7).

2.1.2.1. Reactionary Dentin

In case of mild traumatic injuries or slowly progressing dentinal caries, survival of the odontoblasts is possible. Metabolic by products of bacteria and other toxins can be transmitted via the dentin tubules, and consequently affect the pulp cells. Resident odontoblasts which are responsible for primary dentinogenesis are triggered under the area of damage for establishing the reactionary dentin (7,17).

This newly formed dentin, formed as a reaction to dentinal damage, is deposited at the pulp-dentin interface and shows a highly organized tubular structure similar to primary and secondary dentin. Additionally this structure defends the dental pulp from exogen insults. Although the mechanism of this stimulation is not completely understood, it is thought to occur by stimulating the release of the growth factors present in the dentin (7,18).

2.1.2.2. Reparative Dentin

In rapidly progressive caries lesions, tissue damage caused by cavity preparation and serious dental injuries where the pulp is not exposed, odontoblasts can be destroyed under the affected dentin (19,20). If the dentin-pulp complex has an appropriate metabolic state, odontoblast-like cells can migrate, differentiate to new cells and create new tubular dentin called reparative dentin (21). These cells are also called neo-odontoblast or second-generation odontoblasts. These cells are different from original odontoblast cells. Their origins are still not clear (22). It is stated that the new matrix in the pulp-dentin border usually involves the formation of reactive dentin or fibrodentin under clinical conditions. However these processes cannot be distinguished *in vivo*. Besides, it may not be distinguished from biochemical and molecular aspects (4). In Fig. 1, types of tertiary dentin formation due to different kinds of stimuli are demonstrated (23).

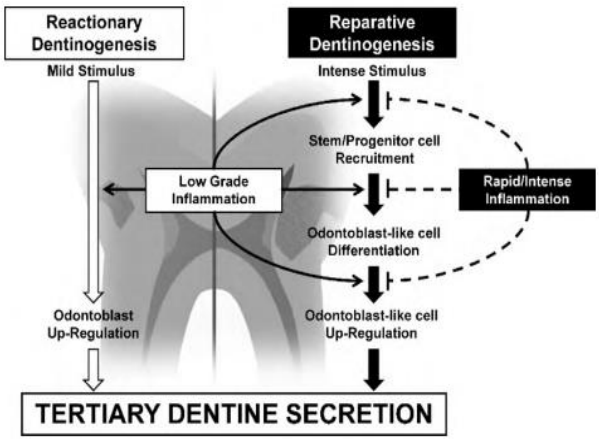


Fig.1: Inflammation and regeneration reaction in dentin–pulp organ (23).

If the pulp is exposed, the remaining pulp may heal itself or may be healed by the help of the pulp capping agents (24–26). If this exposure is caused by decay, the defense mechanism is affected due to the bacterial progression to the pulp and the pulp healing potential is more limited (27). Dental pulp cells' dentinogenic potential is stimulated, progenitor cells proliferate, migrate, and differentiate into odontoblast-like cells which can form reparative dentin and reconstruct the loss continuity at the pulp dentin boundary (28). Injury type and location, age of the tooth, the material used in treatment, and cavity integrity are parameters for the successful outcome in the VPT approach (27).

2.1.3. Stem Cells

Stem cells are functionally undifferentiated cells and have heterogeneous reproductive potential. These cells can divide, transform into different cells, and have self-renewal potential (29). Stem cells are grouped as embryonic and postnatal stem cells (organ-specific, tissue-specific, or adult stem cells) according to the source from which they are acquired. Embryonic stem cells originate from the embryo and can transform into all tissues (30). Adult stem cells are undifferentiated. They usually transform into the cell types in which they are located in. Adult stem cells renew themselves and provide the continuity and repair of the tissue which they belong to. These cells are mostly located in the bone marrow. They also have been found in cord blood, skeletal muscle, digestive system, brain, blood, skin, eye and pancreas, and teeth (31).

The development of the teeth in the embryonic period is originated from ectoderm. Tooth germs are formed from the ectodermal layer, dental papilla, and the tooth follicle are formed from the neural crest. For this reason, the dental tissues contain mesenchymal components which include ectodermal originated neural crest cells (31).

In the tooth development process many types of different stem cells and progenitor cells take part. Some of them are; Dental pulp stem cells (DPSCs) (32), stem cells from human exfoliated deciduous teeth (SHEDs) (33), stem cells from the apical papilla (SCAPs) (34,35), periodontal ligament stem cells (PDLSCs) (36) and Dental Follicle Stem Cells (DFSCs) (37).

Dentin-pulp complex has a natural regenerative potential in tertiary dentin formation. Odontoblasts can survive in mild stimulus such as dental caries, erosion or

wear and secrete the reactionary dentin. If the damage is more severe for example in extended dentin caries and trauma, primary odontoblasts may loose viability (7,38). In this situation, the dental-pulp complex allows differentiation of the stem cells and the development of reparative dentin. Postnatal pulp has different types of progenitor/stem cells that enable the release of reparative dentin (39).

The DPSCs are multipotent mesenchymal stem cells which have a high proliferation capacity with an ability of cloning and do not abolish after eruption period. These cells were first described by ***Gronthos et al.*** and isolated from the third molar. It has been shown that these cells transform into osteoblastic cells and chondrocyte cells *in vitro* and to odontoblastic cells *in vivo* (32). DPSCs are activated by the release of growth factors after tooth decay and take part in the development of repair tissue, while the regeneration of the dentin-pulp complex (40).

2.1.4. Biomolecules

Dentin deposition as a reaction to exogen insults is regulated by lots of bioactive molecules. Recent studies mostly focused on transforming growth factor (TGF) (41), bone morphogenetic protein (BMP) (42), insulin-like growth factor (IGF) (43), fibroblast growth factor (FGF) (44) and fibronectin (45). Underneath the caries; these biomolecules are released by acidic situations and stimulate the odontoblast for reactionary dentin secretion locally (16).

Fibronectin is a glycoprotein located in blood plasma and tissues. It is stated that it involves cellular reactions like adhesion, chemotaxis, and cytodifferentiation (46). Fibronectin is reported to be a key molecule that regulates adhesion of matrix secreting cells to the substrate like predentin, dentin, cementum. Also it is localized in odontoblast layer and regulates odontoblast polarization and cytodifferentiation. Another role of fibronectin isto induce the cytodifferentiation of odontoblasts by binding TGF-β and dentin matrix secretion (47).

Bone morphogenetic proteins (BMPs) are cytokine groups and they are members of TGF-β superfamily. BMPs also promote odontoblast differentiation and dentin formation. The most important members of this group are BMP-2, BMP-4, and BMP-7 which involves in the reparative dentinogenesis process (48,49).

TGFβ is another important growth factor that mediates tissue repair, proliferation, differentiation, mineralization of dental pulp stem cells. In some studies; TGF-β1, TGF-β3, have reported to increase the odontoblast activity (50,51).

Bulk of dentin matrix is mostly collagen, in which non-collagenous proteins are involved. They also help cytodifferentiation, matrix secretion, and mineralization. For example dentin phosphoproteins and sialoproteins are involved in the mineralization process (52).

2.2. Vital Pulp Therapies

During the tissue repair process, cells and tissues mimic the mechanism of tooth development so it is possible to evaluate the biologic process of VPT's (4). Vital pulp therapy (VPT) is a treatment that is used for conserving pulp tissue affected by mild caries, traumatic injuries, and iatrogenic interventions. VPT is especially crucial in the young tooth which has incomplete apices. VPT is recommended to perform in young patients due to higher healing capacity beside older patients (53,54). However this does not mean that old patients can not take advantage of this treatment. While considering the microbial contamination, the healing ability of the pulp; preservation of the permanent tooth could be applied by using VPT (55).

In root-canal-filled teeth, resistance to mastication forces is lower than the healthy teeth, especially molars. Hence, this explains why it is essential to apply VPT whenever possible (56,57). The status of the pulp tissue is also an important factor to consider before decision of the type of treatment of choice. Formerly, it was accepted that VPT treatment should only be applied in a tooth with signs and symptoms of reversible pulpitis (58). However it is impossible to determine the accurate outcome of treatment, since signs and symptoms related to pain and response to sensibility tests do not always reflect the definite condition of the pulp (59,60). Studies have reported teeth with signs and symptoms of irreversible pulpitis which have been treated with a VPT revealed successful outcomes (61–64). It is stated that bleeding in the site of pulp exposure is a more accurate sign to show pulp inflammation (61). If the bleeding is difficult to control in the exposure area, this shows that inflammation extends to deeper tissues and treatment protocol should be also extended to DPC or pulpotomy (65). For the maintenance of pulp vitality adequate blood supply is required (66). Also healthy periodontium is another important factor for

achievement of clinical and radiographical success. It is not indicated to perform VPT to the teeth with severe periodontal findings (67).

An efficient and a thight coronal seal should be established to have better clinical outcomes after VPT. Poor coronal seal could worsen the prognosis after the treatment due to the microleakage of the bacteria. (68). Pulp hemostasis is necessary in order to achieve an optimum outcome following VPT (61). Sterilized cotton pellets which absorbe water or saline can be used for hemostasis with the compression on the pulp tissue mechanically. Cavity disinfection is another important factor to be considered (69). Sodium hypochlorite (NaOCl); should be used for control of hemorrage, removal of coagulum and dentin chips, and cavity disinfection, all of which aid in the formation of a hard tissue barrier at the site of exposure (70).

Pulp capping agents have remarkable effects on the success of VPT. These materials are expected to be biocompatible, noncytotoxic, antibacterial, and have proper physical and chemical mechanical features (71).

2.2.1. Indirect Pulp Capping

Indirect pulp capping (IPC) is applied in primary and permanent teeth when carious dentin gets too close to the pulp, covering the closest area with a biocompatible material to avoid pulp exposure when there are no signs and symptoms of pulp degeneration (72). This treatment's success rate is reported as very high for permanent teeth. Approximately 90% of success was reported without any symptoms (1,3). In the IPC treatment technique, the aim is to preserve vitality of primary odontoblasts which also stimulate reactionary dentin formation at the pulp-dentin border (3,7). But in some cases due to the depth and severity of the caries process; primary odontoblasts may be destroyed and reparative dentin and the reactionary dentin are formed simultaneously (3). One of the major objectives in applying bioactive liners is to induce the odontoblasts in the dentin-pulp complex to form tertiary dentin (73).

New reports suggest that rather than all caries removal; incomplete caries removal techniques are considered to preserve the vitality of the pulp. IPC can be applied mainly in two ways: incomplete caries removal without reentry and two-step excavation technique (stepwise technique). In stepwise technique, carious dentin over the pulp remains and only excavation of carious dentin at the enamel-dentin border is performed

at the first visit. The objective in this treatment is to remove the number of bacteria, change the cariogenic environment, and slow and/or arrest caries development. In the second appointment (three to six months), the cavity is reentered, all carious tissue is removed, a pulp capping material is placed and this is followed by a permanent restoration. In this time interval tertiary dentin is expected to form. It is important to seal the margins appropriately in both steps to avoid microleakage. While performing this technique pulp exposure may occur even though residual carious dentin gets harder, the thickness may not be changed (72,74).

In a recent systematic review of ***Clarkson et al. (2008)*** emphasized that partial removal should be applied for deeper caries to avoid the exposure of the pulp (75). Another published systematic review by ***Schwendicke et al. (2013)*** reported that incomplete caries removal without reentry procedure reduces the risk of exposure of the pulp and occurence of postoperative symptoms when compared to stepwise removal technique (76).

As long as the optimum amount of carious dentin which should be left in the cavity is not prescribed to avoid the post-operative pulpal symptoms; removing all peripheral carious tissues in dentin wall for obtaining tight marginal adaptation and removing as far as possible caries next to the pulp, paying attention to expose the pulp are essential to obtain good results (74).

Unlike previous beliefs; in primary teeth with reversible pulp inflammation, IPT can be used as a treatment option. However diagnosis, clinical and radiographic examination, and proper leakage-free restorations are the keys factors for success (4). IPC success in primary teeth is well-documented (77–80). For long term studies IPT in primary teeth was reported to have a more successful outcome rather than pulpotomy treatments (81–83). Due to the evidences of IPT success in primary teeth, it is recommended that the IPT technique can bu used in deep decayed primary teeth without any signs and symptoms of irreversible pulpitis or necrosis and should provide leakage-free restoration to increase the success rate (4).

2.2.2. Direct Pulp Capping

Direct pulp capping (DPC) is a treatment defined as the placement of a biomaterial directly on the exposed pulp, when a pinpoint mechanical exposure is encountered during cavity preparation or traumatic injury, to promote reparative dentin

formation and maintenance of pulp vitality (72). For a successful outcome, the size of the exposure site should be similar to a pinpoint at diameter, and the tooth should be asymptomatic whereas the isolation could be done appropriately (4).

When the primary odontoblasts are demolished at the pulp exposure area, uninfected vital pulp is expected to produce reparative dentin by stem cell recruitment and differentiation (3). Induction of stem cells to differentiate into odontoblast-like cells are occurred by the release of growth factors like transforming growth factor (TGF). Pulp capping materials like CH and mineral trioxide aggregate (MTA) placed on the exposure site induce the growth factor release from the dentin matrix so reparative dentin is formed to preserve the vitality of the pulp (4,84).

In some studies it was said that; DPC must only be applied for recent iatrogenic exposure or pulp is exposed to trauma (25,58,85,86). However ***Aguilar and Linsuwanont (2011)*** reported that the success rate in permanent teeth with a carious pulp exposure which were treated with DPC was 87.5 to 95.4% (55). These results were similar to the rates found for treatment outcomes after iatrogenic pulp exposures (70 to 98%) (56). To achieve a successful result after DPC; elimination of the microorganisms in the cavity is accepted as the critical and most important factor (56,58,85). Residual bacteria which are a result of leakage from the cavity margins are well accepted to decrease the success rate. Leakage free restorations, achieved by rubber-dam use and aseptic techniques are the key points for the successful outcome (65). ***Matsuo et al. (1996)*** evaluated the tooth type, age, spontaneous pain existence, diameter of pulpal exposure, degree of bleeding for the success of treatment. These factors did not affect the success rate significantly. Only lesser bleeding was found to promote pulp healing (61).

In primary teeth DPC is not recommended when pulp is exposed by caries (6). DPC is indicated exclusively when the pulp is exposed very little mechanically during cavity preparation or due to a traumatic injury (72). Nevertheless, DPC treatment in primary teeth does not reveal successful results as in permanent teeth. In case of failure internal resorption or dentoalveolar abscess may occur. But still, DPC must be considered as a treatment option for primary teeth to preserve the pulp vitality (4). ***Caicedo et al. (2006)*** used DPC or pulpotomy in 11 primary teeth by applying MTA. After restoration they took radiographs of the teeth after 1 month and five months just before the extraction.Teeth showed no root resorption, no furcation radiolucencies also no mobilitiy

and abcess even though different histological responses were noted. They concluded that MTA may be favourable material for pulp capping and pulpotomies in primary teeth (87).

2.2.3. Pulpotomy

Partial pulpotomy is a procedure defined as, the removal of the inflamed pulp tissue underneath the exposure site approximately one to three millimeters or deeper which is extended to reach the healthy tissue (72). Partial pulpotomy in traumatic exposures is named as Cvek pulpotomy (54).

Hemorrhage in the pulp can be controlled by application of antibacterial agents such as NaOCl or chlorhexidine digluconate (88,89) Following hemorrhage contol, bioactive materials like CH or calcium silicate-based materials can be used similar to DPC (8).

This technique has some superiorities to DPC. In this therapy it is possible to remove inflamed pulp tissue more accurately. Also the procedure provides a wider area for the lining agent so that the ability of the material to seal the cavity is more profound. The success rate is reported 93-96% for partial pulpotomy (90,91).

Full pulpotomy is the elimination of the whole coronal portion to preserve the vitality of the remaining pulp (92). If it is thought that inflammation has extended deeper levels than the superficial pulp tissue; full pulpotomy should be considered. The mechanism at the cellular and molecular level while the formation of the dentin bridge on pulpotomy procedure is like in DPC. Same as the other vital pulp therapies, hemostasis should be applied and a biomaterial should be located on the residual pulp tissue (7).

In the primary teeth, the agents and/or materials used in the conventional pulpotomy procedure were formocresol, ferric sulfate and CH in the practice for many years (93–95). Recently; MTA has been introduced and widely applied for pulpotomy and its success rate has been found very high compared to other conventiomal materials (4,96–98). There are several materials and techniques which are indicated an succesfully used in vital pulp treatments. These are CH (93), calcium silicate-based materials (99) electrosurgery (100), laser (101), freeze-dried bone (102), bone morphogenetic protein (48), osteogenic protein (103).

2.3. Materials Used in Vital Pulp Treatment

Calcium hydroxide (CH) is the first documented by ***Hermann*** in 1930 for exposure area application (104). Until that day different CH types such as powder form, paste, and cements used widely in dentistry for vital pulp therapies and considered as the gold standard. CH's are used for reparative dentin formation, protect the vitality of the pulp, stimulate mineralization, and antibacterial effects (105,106).

In 1963; Dycal (Dentsply Caulk, Milford, DE, USA) which is calcium-based cement first used in a clinical study. It's success rate found 85% compared to the CH and saline mixture (80% success rate) (107).

Zinc oxide eugenol is also used for DPC. But it was reported that Zinc oxide eugenol could not induce dentin bridge formation and caused chronic inflammation, high leakage, and also was found toxic to the cells (108,109).

Glucocorticoid and antibiotic combinations were used in the 1970s to avoid pulpal inflammation and decrease pulpal pain. However wound healing was found insufficient also pulp necrosis has been reported. Because of these drawbacks usage of steroids abandoned (110,111).

During the 1990s; mineral trioxide aggregate (MTA) was first introduced by Torabinejad and White (112). It is originally a hydraulic Portland cement which is a calcium silicate-based material. Hithero, MTA has been used broadly for its high clinical success rates, more recently new MTA-like materials have been launched in the dental market (8).

2.3.1. Calcium Hydroxide

Calcium hydroxide (CH) based agents have been in clinical practice for many years and are still accepted as gold standard for their antibacterial characteristics and hard tissue formation ability while applying vital pulp therapies (13,61,113). CH has the capability to induce the dental pulp cells for recruitment, migration, proliferation, and mineralization (14).

The mechanism of action of CH is simply based on the generation of a chemical wound due to the high pH of the material. This process is like the healing process of soft tissue wounds. Initially CH causes superficial necrosis in the tissue. This is also called a

coagulation necrosis. This superficial necrosis causes irritation and thus vital pulp tissue is stimulated for reparative dentin formation. This formation occurs through cellular migration and differentiation, ECM secretion, and mineralization (114). ***Lu et al. (2008)***; reported after seven days, the inflammatory reaction was seen by the infiltration of neutrophils and mononuclear cells. By dilated blood vessels, pulp tissue was disorganized. Thirty days later, inflammatory reactions began to fade and secretory potential cells arrived at the location of tissue repair (115). These events are caused by the mechanisms of biologically active molecules and signaling pathways (16).

Many studies have been carried out which showed CH's effect on these biomolecules such as; increasing the rate of TGFβ secretion from dental pulp cells (116), inducing mineralization by modulating the expression of bone sialoprotein and osteopontin (117,118), increasing the level of interleukin-1 beta (IL-1β) expressing cells, TGFβ-expressing macrophages and BMP levels (119), expression of fibronectin in dental pulp cells (120,121), activation of notch signaling genes which are believed to take part in cell differentiation (122), elevation of alkaline phosphatase enzyme in the odontoblastic and subodontoblastic cell layers (123), when in contact to CH.

However antimicrobial properties of CH, has limited effects due to a restricted solubility and diffusibility. Thus the material is efficient only at external levels. It can be said that CH' s success is limited to minimal bacterial invasion, if contamination reaches deeper levels, the success rate decreases (124).

DPC's clinical success rate primarily depends on the formation of a dentin bridge. It is stated that after using CH on monkey teeth; 89% of dentin bridges had tunnel defects which may lead to failing a long time barrier and bacterial invasion (125). Also long term solubility (126), microleakage (127), lack of adhesion to the dentin (128) are other disadvantages of CH.

2.3.1.1. Aqueous Calcium Hydroxide

Formerly, the CH powder was directly placed on the pulp tissue. After this direct contact, pulpal fluid and powder mixture makes a paste formation. Recently this technique is abandoned. In some studies it is reported that this direct contact caused inflammatory response (129) after that aqueous CH paste was used. This is the mixture of CH with saline or water before the application. Premixed types like UltraCal XS

(Ultradent Products, South Jordan, UT, USA), Calcicur (Voco, Cuxhaven, Germany) are available. Due to the disadvantages of aqueous CH such as difficult setting properties and slow resorption after placement of medicament, tunnel defects in dentin which causes microleakage; CH-based cements were produced (55).

2.3.1.2. Calcium Hydroxide-Based Cements

In the 1960s a cement type of CH was developed to overcome disadvantages of aqueous CH and improve setting characteristics. Dycal is the most widely used and popular CH-based liner. It consists of one tube of catalyst and one tube of base which should be mixed by the ratio of 1:1. The catalyst includes calcium hydroxide, N-ethyl-o/p-toluene sulfonamide, zinc oxide, titanium dioxide, and zinc stearate. The base contains 1,3-butylene glycol disalicylate, zinc oxide, calcium phosphate, and calcium tungstate. There are some other brands in the dental market. One of them is Life (Kerr, Orange, CA, USA) mainly the same ingredients with some differences and different CH-based pastes are available in the dental market today (Fig.2) (8).

Fig.2. Different brands of CH-based cements.

Ideal cavity liners should not be soluble in water and other organic and inorganic fluids, resist masticatory forces, and should support the overlaying restoration. However, conventionally used CH liners have very high solubility properties, while having no ability for adhesion to dentin. Also manipulation and application of these materials are difficult (130). To avoid these problems, new resin-based cavity liners have been produced which contain CH (Fig. 3). Regarding conventional CH, resin-based materials have better physical and handling characteristics, and much more resistant to solubility (130,131).

Fig. 3. Different brands of resin modified CH-based liners.

Although CH-based materials are widely used in the present day, they have some disadvantages such as causing porosities in hard tissue, degradation due to acid etch procedure, stimulation of excessive dentin formation which may cause calcifications in the pulp chamber, lack of adhesion, and solubility with the tissue fluids as it mentioned before (12,132). Due to these problems in CH-based materials, new materials have been produced.

2.3.2. Calcium Silicate-Based Materials

2.3.2.1. Hydraulic Materials

2.3.2.1.A. Mineral Trioxide Aggregate (MTA)

Mineral trioxide aggregate (MTA) was first established at Loma Linda University for filling the root apices and seal lateral root perforations in the 1990s (133–136). Because this material is hydraulic and sets in the presence of water; it can successfully be used in these areas (137).

In 1998 the first marketed MTA is ProRoot MTA Gray (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA) (Fig 4). This material is mainly composed of Type I Portland cement (75%), which consist of 55 wt% tricalcium silicate ($3CaO•SiO_2$), 19 wt% dicalcium silicate ($2CaO•SiO_2$), 10 wt% tricalcium aluminate ($3CaO•Al_2O_3$), 7 wt% tetra calcium aluminoferrite ($4CaO•Al_2O_3•Fe_2O_3$), 2.8 wt% magnesium oxide, 2.9 wt% sulfate and 1.0 wt % free calcium oxide. Besides Portland cement; MTA consists of 20% bismuth oxide and 5% calcium sulfate dihydrate which is for providing radioopacity and setting modification (8).

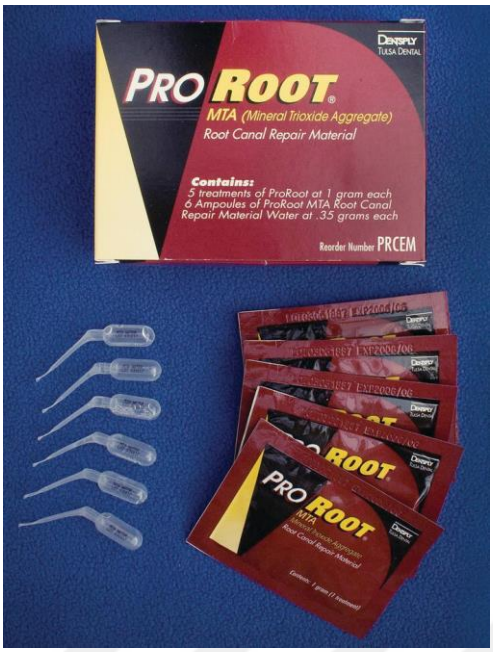


Fig. 4. ProRoot MTA Gray.

In 2002 ProRoot MTA White was launched (Fig. 5) This material's difference from gray MTA is the elimination of iron components which caused esthetic drawback; additionally calcium silicate content is higher in White MTA (8).

Fig. 5. ProRoot MTA White and difference from ProRoot MTA Gray.

More recently different types of MTA products were available in the worldwide dental market (Table.1) (8). Some examples of various MTA brands are shown in Fig. 6

Table 1: Examples of MTA products and brand names (8).

Product	Brand Name
MTA Angelus	Angelus, Londrina, Brazil
ProRoot MTA White / Gray	Dentsply Tulsa Dental Specialties, Johnson City, TN, USA
MTA Plus White	Prevest Denpro, Jammu, India
Tech BioSealer MTA	Isasan S.R.L., Rovello Porro, Italy
NEX MTA	GC, Tokyo, Japan
EndoCem MTA	Maruchi, Gangwon-do, Korea
Grey MTA Plus	Avalon Biomed, Bradenton, FL, USA
MM-MTA	Micro Mega SA, Besançon, France
MTA+	Cerkamed PPH, Wojciech Pawlowski, Nisko, Poland
MTA Caps	Acteon, Merignac, France
Trioxident	VladMiVa, Belgorod, Russia
Ortho MTA	BioMTA, Daejeon, Korea
Endo-Eze MTA	Ultradent Products
Aureoseal M.T.A.	Ogna Laboratori Farmaceutici, Muggiò, Italy

Fig. 6. Different brands of MTA materials.

Mainly MTA mechanism is very similar to the CH because after hydration of MTA; CH is produced as a by-product which causes superficial necrosis when placed on the pulp. the following reaction shows the creation of calcium silicate hydrate gel and $Ca(OH)_2$ after hydration reaction when mixing the MTA powder and water (8).

$$2[3CaO{\bullet}SiO_2]+6H_2O \rightarrow 3CaO{\bullet}2SiO_2{\bullet}3H_2O+3Ca(OH)_2$$

$$2[2CaO{\bullet}SiO_2]+4H_2O \rightarrow 3CaO{\bullet}2SiO_2{\bullet}3H_2O+Ca(OH)_2$$

MTA has similar properties with CH-based materials regarding stimulation of hard tissue formation, mainly due to their capability of releasing $Ca(OH)_2$. Furthermore, MTA has a good sealing capacity and was shown to be a bioactive and biocompatible dental material (138–140).

It is reported in many studies that, dentin bridge formation is more uniform and thick, also inflammatory response is less when compared to application of CH (140,141). ***Leye Benoist et al. (2012);*** evaluated MTA and CH for dentin bridge thickness. At 6 months of control, the success rates and the mean thickness of newly dentin formation were found alike radiologically (142).

In terms of antibacterial effect; it is reported that MTA is effective is on some types of facultative bacteria; but on strictly anaerobic bacteria the material did not show any antibacterial effect (143). Also in some studies MTA showed less antibacterial efficacy when compared to CH and zinc oxide eugenol (144,145).

In different animal studies which evaluated the outcomes of various pulp capping procedures; it was stated that MTA showed greater pulp healing ability compared to CH regarding the development of dentin bridge and rate of inflammation (146–148). MTA was also found superior to Dycal in terms of new hard tissue formation and pulpal inflammation in human studies (149–151). These outcomes may be related to MTA's better-sealing ability, creation of a more alkaline environment, and biocompatibility (150). MTA produces CH with hydration reaction; eventually leading to release of calcium and hydroxyl ions which stimulate the hard tissue formation (8).

Although MTA has many advantages, disadvantages such as tooth discoloration especially at the coronal area, difficult handling characteristics, and need a long time for setting are reported in different studies (152–158). Setting time differs in various studies. 50 minutes (153), less than 4 hours (159), and 70 minutes for initial and 175 minutes were reported for final setting times (160). MTA Gray’s setting time (165 minutes) was reported shorter compared to MTA White (140). Prolonged setting requires two appointments; first visit for MTA application and second visit for permanent restoration. In between two appointments MTA is expected to be hardened. This may cause both bacterial contamination and prolongs the duration of treatment. It may be possible to perform treatment in one appointment due to a shorter setting time and this creates an advantage for long term prognosis. Another disadvantage is difficulty of handling related to coarse particles in the powder, which cause a "sandy feeling" mixture. The powder consists of large particles so that the water can not dissolve evenly. The uneven distribution of water makes the condensation harder. These properties like handling and setting time are related to particle size and shape of MTA powder.

MTA White is recommended while using at esthetic zone because tooth discoloration cases were reported when gray MTA was applied for DPC. (155). However in some studies white MTA is reported to cause tooth discoloration even after application for endodontic treatments (156,157). ***Ioannidis et al. (2012);*** compared tooth discoloration after application of white MTA Angelus and gray MTA Angelus. They reported that the discoloration level was found higher for gray MTA. After 1 month Gray MTA caused clinically perceptible discoloration. Discoloration occurred by white MTA became visible after 3 months. Both materials decreased the lightness, redness and yellowness levels. They stated that the application of gray MTA in the esthetic regions of the teeth should be avoided and also white MTA should be used carefully (158).

Different factors were stated for tooth discoloration while using white MTA such as sodium hypochlorite contact (161), blood contamination (162,163), light, and oxygen presence (164,165). Also the presence of radioopaque bismuth oxide may be a cause of discoloration (164). Tooth discoloration mechanism is not exactly understood and further studies should be carried out.

2.3.2.1.B. Modified MTA's and MTA-Like Materials

Modified MTA's have been produced in order to overcome the disadvantages of MTA material. The main purpose of production of these materials was to reduce the setting time by modification of particle sizes of the powder. Calcium oxide was added in Angelus White MTA (Angelus, Londrina, Brazil) instead of calcium sulfate which decreased the setting time. Angelus White MTA's setting time is approximately 15 min. (166). Also for reducing the setting time, calcium carbonate was incorporated into MM-MTA (Micro Mega SA, Besançon, France), calcium chloride, and montmorillonite into Tech BioSeal MTA (Isasan S.R.L., Rovello Porro, Italy). MTA Plus's powder is more finely ground (8).

In 2006 MTA-like materials were produced. These materials do not contain Portland cement. Synthetic calcium silicates are the primary ingredients of these materials. They also do not contain aluminum components. Metal released from set materials depends on the origin of the calcium silicates (167). For example Angelus MTA and MM-MTA are derived from Portland cement; they contain a huge amount of aluminum and also a trace amount of chromium, arsenic, cadmium, and beryllium. DiaRoot Bioaggregate (DiaDent Group International, Cheongju-si, Korea) have synthetic

calcium silicates. No metal groups were found in this material except for a small amount of aluminum. BioAggregate which was introduced to the dental market in 2006, contains tricalcium silicate, dicalcium silicate, amorphous silicon oxide, and calcium phosphate monobasic (calcium dihydrogen phosphate). From setting (hydrating) reaction of calcium silicates, CH is formed and calcium phosphate in this material reacts with part of that. During the reaction process, water and hydroxyapatite is produced. This water accelerates the hydration reaction. On the other hand silicon oxide interacts with this CH which also affects the setting time. It is stated that setting time is 4 hours for DiaRoot Bioaggregate also its antibacterial effect was found similar to Dycal but lower than zinc oxide/eugenol (168).

Biodentine (Septodont, Lancaster, PA, USA) is also a bioactive material and by releasing calcium and hydroxyl ions; stimulates the formation of hard tissue (169). Biodentine is a capsulated form of tricalcium silicate material introduced in 2009 (Fig.7). Its main ingredients in the powder are tricalcium silicate, dicalcium silicate, calcium carbonate and oxide, and zirconium oxide as radioopacifier; the liquid contains calcium chloride which is used for acceleration of the hydration and modified polycarboxylate. These materials in the liquid provide a shorter setting time approximately 10 to 12 minutes. Polycarboxylate helps to reduce the required water and provides better handling. Due to the capsule form of the material application errors are reduced and it is possible to create a more suitable setting time. Calcium carbonate also leads to a faster setting time by acting as a nucleation site and in addition to these the powder part has finer particles with larger surface area. The surface area of Biodentine is stated approximately 2.8 fold wider than MTA Angelus White. All of these features affect the shortening of setting time (166). ***Nowicka et al. (2013)*** reported that histological evaluation after 6 weeks after the application of Biodentine; full dentin bridge formation was observed and no inflammation of the pulp was evident. They also found odontoblast and odontoblast-like cells under the osteodentin (170).

Fig. 7. Biodentine

EndoSequence BC RRM (Brasseler USA, Savannah, GA, USA) which consists of tricalcium silicate, dicalcium silicate, calcium dihydrogen phosphate, tantalum pentoxide, zirconium oxide, calcium hydroxide was produced in 2009. EndoSequence has a premixed-syringeable form and no need to be mixed with water. The setting time is stated for approximately 2 hours. Lately, also BC RRM-Fast Set Putty was introduced with a faster setting time about 20 minutes in 2014 (171). Cytotoxicity levels, biocompatibilities, ability to induce proliferation of the cells of the dental pulp, and dentin bridge were found similar to ProRoot MTA and MTA Angelus (171–174).

2.3.2.2. Resin Modified MTA

TheraCal LC (Bisco, Schaumburg, IL, USA) is a light-cured calcium silicate-filled base/ liner material *(Fig.8).* It contains calcium oxide, calcium silicate particles (45% type III Portland cement), strontium glass, fumed silica, barium sulfate, barium zirconate, and resin consisting of Bis-GMA and polyethylene glycol dimethacrylate (9).

Fig.8. TheraCal LC

Gandolfi et al. (2012); reported TheraCal's water sorption was found significantly lower than ProRoot MTA but higher than Dycal. They recommended that polymerization depth should not be more than 1.7 mm to avoid dissolution (9). ***Hebling et al. (2009)***; assessed the cytotoxic effects of TheraCal and concluded that TheraCal is well-tolerated by odontoblast cells (175).

2.4. pH of Dental Materials

In chemistry, pH is a scale to measure the acidity or alkalinity of water-based solutions and also called the power of hydrogen. The pH value of a substance directly depends on the ratio of hydrogen ion [H^+] and hydroxyl ion [OH^-] concentrations. If the H^+ concentration is higher than the OH^- concentration, the solution is acidic; that is, the pH value is lower than 7. If the OH^- concentration is higher than the H^+ concentration, our substance is basic; that is, the pH value is greater than 7 (176).

OH^- ions released from CH and calcium silicate materials; elevates the pH of the environment to alkaline levels so creates an antibacterial effect (177). Only a few bacteria may be vital in pH 12.5. The environment that CH creates can reach this pH level. Also; elevated pH makes acid neutralization created by resorptive cells so regulates the acidity created by inflammation mechanism (178). ***Okabe et. al. (2006)*** stated that an increase in pH stimulated the increase in BMP-2, alkaline phosphatase levels, and the formation of calcified nodule (179). If the pH level is higher than 9, the cellular membrane enzymes of microorganisms are inactivated (reversibly or irreversibly) as a result their biological activities are affected (180).

2.4.1. pH Evaluation Tecniques

Simple pH evaluation can be carried out with the aid of pH papers which create color change after dipping into the solution. After turning into a specific color; the scale shows us if the material is basic, acidic, or neutral. However this method is limited in terms of reliability and does not give any quantitative result. For more accurate and quantitative results pH meter should be used for laboratory tests (181).

A pH meter is a scientific tool that evaluates the hydrogen ions in water-based samples. This device shows the acidity or alkalinity of solutions and demonstrates the level of pH (182). This evaluation is occurred by an electrical potential difference of pH electrode (glass electrode) and a reference electrode. Because of that it is also called potentiometric pH meter. This difference depends on the acidity or pH of the water samples. Sometimes the device can be produced with combination electrode. By immersion of these electrodes into the solution, an electrical circuit is completed, in which there is a potential difference created and detected by the voltmeter. Calibration is applied before every use with standard pH solutions, to get more accurate results (183). pH meters are used in different areas such as, agriculture, swimming pools, wine brewing, manufacturing, health care, laboratory tests, etc (184).

2.5. Calcium Release

Calcium hydroxide is a material used in dentistry for various purposes since the first half of the past century. With the application of calcium hydroxide, calcium ions (Ca^{2+}) are released and bioactive events occur in the surrounding tissue. Ca^{2+} induces hard tissue formation (185). Ca^{2+} ions induce hard tissue formation by the formation of odontoblast-like cells, proliferation, migration, and odontogenic marker expression and mineralization (186). ***Mizuno and Banzai (2008)*** conducted an *in vitro* study; cells of human dental pulp were exposed to elevated levels of Ca^{2+} by applying CH, this induced fibronectin synthesis in pulp tissue which is involved in differentiation and secretion process (45). ***Rashid et al. (2003)*** stated in culture conditions elevated Ca^{2+} levels from CH, increased the mRNA levels of BMP-2, and osteopontin (118).

2.5.1.Calcium Release Evaluation Tecniques

Various techniques are used to determine the calcium ions in solutions. Although calcium probe is used in some studies (9,187,188); in recent years it has been

accepted that spectrometric methods give more accurate results. For evaluating elemental analysis; the most widely used technique is the atomic spectroscopy. Spectroscopy is defined as the measurement and interpretation of the electromagnetic radiation absorbed or emitted during the transition of atoms, molecules, or ions in one sample from one energy level to another. Different types of atomic spectroscopic methods are available for detecting elements which are used in different types of scientific areas. a) Atomic emission methods b) Atomic absorption methods, c) Photoacoustic detection, d) Atomic fluorescence methods, e) Atomic ionization methods (189).

Atomic absorption spectroscopy (AAS) is one of the most common methods used for the determination of elements. Atomic absorption spectrometry is used in biological, clinical, and environmental research laboratories and analytical laboratories for routine analysis. The tool is relatively easy to use. In an atom launcher (automizer), an element can rise to orbitals with high energy requirements. This amount of energy (or wavelength) is something specific to a particular electron transition in a particular element, and in general, wavelength belongs to only one element. This gives the technique element selection feature. If the amount of energy (power) given to the environment is determined and the amount of energy remaining the other side (detector) can be measured, transition amount can be calculated and the signal corresponding to the concentration of the measured metal can be obtained (190).

AAS is based on the principle of measuring the absorption of electromagnetic light by gaseous element atoms at high temperatures. To analyze an element with AAS, it must first become neutral, then vaporized, and then be dispersed in the path of the electromagnetic beam from a source. Atoms that absorb light move from the basic energy level to unstable excited energy levels and the amount of absorption depends on the number of atoms at the basic level. The most important components of atomic absorption spectrometers are the light source (hollow cathode lamp) that emits the radiation absorbed by the analysis element, the atomizer where the sample solution is transformed into atomic vapor, the monochromator where the studied wavelength is separated from the other wavelengths and the detectors where the light intensity is measured. Hollow cathode lamps are the most used light sources in the atomic absorption spectroscopy method. The atoms stimulated by the voltage created in the lamp radiate at the wavelength specific to the cathode element while returning to the basic energy level. For each element examined,

the hollow cathode lamp specific to that element must be placed on the spectrometer (191).

2.6. Studies On The Evaluation of pH and Calcium Release of Calcium Hydroxide and Calcium Silicate Materials

Duarte et al. (2007) evaluated the NuCap, Hydro C, Life, and Ultrablend Plus materials with CH content for calcium release and pH analysis. Samples were evaluated for 3, 24, 72, and 168 hours with pH-meter and atomic absorption spectrophotometer. It was reported and concluded that, all samples released calcium and hydroxyl throughout the study periods except Ultrablend Plus. Even though Ultrablend Plus had significantly the highest calcium release; it did not release hydroxyl ions (192).

Ghazvini et al. (2009) evaluated the Portland cement, ProRoot MTA, calcium-enriched mixture (CEM) cement for calcium ion release, and pH values during 1, 3, 24, 48 hours, 7 and 28 days. Atomic absorption spectrophotometer was used to measure released calcium ions. For evaluation of pH rates; Metrohm 744 pH meter was used. pH and calcium ion release rates were calculated, both periodically and cumulatively. All materials created an alkaline pH. When examining cumulative results, at the end of the study, tested materials' pH or calcium release values showed no significant difference. They concluded that all materials released large amounts of calcium and created alkaline pH so this environment could stimulate hard tissue calcification process (193).

Gandolfi et al. (2012) investigated and compared the calcium release and pH levels of TheraCal, ProRoot MTA and Dycal materials. Magnetic stirrer using a multiparameter laboratory meter connected to a calcium probe or a (selective) temperature compensated pH probe/electrode was used. The measurements were carried out after 3 and 24 h, and 7, 14, 28 days. TheraCal released significantly more calcium than Dycal and ProRoot MTA during the study. It was observed that, the amount of released calcium levels dropped gradually for all of the materials. TheraCal alkalinized the medium initially to approximately pH level of 10–11 in between 3 hours–3 days, and 8–8.5 in between 7 hours–28 days. In conclusion they summed up the high calcium release and alkaline pH forming ability of TheraCal material could induce apatite formation and differentiation of odontoblasts to form new dentin (9).

Gandolfi et al (2013) evaluated Biodentine and ProRoot MTA materials for calcium release and pH at 3, 24 hours, 3, 7, 14, 28 days. The pH was evaluated by a selective temperature-compensated electrode connected to a multi-parameter laboratory meter and calcium ion release with calcium probe. Cumulative calcium release was calculated separately. Both dental materials created an alkaline environment. The alkalinity was shown to decrease over time, however still maintained towards the end of the study period. Both materials released calcium ions but the amount of calcium ions decreased over time. At short and long term (3 hours and 28 days) Biodentine exhibited excessive calcium ion release. However, the amount of calcium released by ProRoot MTA stayed constant. It was concluded that, Biodentine showed greater ability to release calcium and extended alkalinizing activity with the help of the wet biointeractive surface which is created by open porosities. With these properties and fast-setting time, Biodentine could be alternative choice to conventional MTA materials (188).

Gandolfi et al. (2014) compared MTA Plus, ProRoot MTA, and Dycal for calcium release and pH levels. The pH rates were evaluated by a pH probe connected to a multiparameter meter and calcium release results were evaluated by calcium ion-selective probe at 3 hours and 1, 7, 14, and 28 days. Cumulative calcium release was calculated. MTA Plus was mixed with both distilled water and the gel provided with the powder. At 3 hours, all materials created an alkaline pH, the maximum value was achived with MTA Plus mixed with gel (approximately 12.0). During 28 days, the pH decreased for all materials due to the gradual reduction in the amount of hydroxyl ions released. At the end of 28 days, Dycal revealed the highest pH (9.8), whereas ProRoot MTA had the lowest pH value (7.1). After 3 hours, the amount of calcium ions released were maximum for MTA Plus mixed with water or gel, also minimum for Dycal and ProRoot MTA materials. During the study period the concentartion of released calcium ions decreased for all materials. At the end of the study, MTA Plus with gel had the highest amount of calcium release compared to other materials. As conclusion it was said that, higher calcium release and alkaline pH of MTA Plus when compared to ProRoot MTA can be explained by the finer calcium silicate particled contained in MTA Plus's powder and could be used as an alternative to conventional MTA materials (194).

Hassan et al. (2015) compared the pH changes and release of calcium ions Biodentine and White ProRoot MTA at 1, 7, 14, and 28 days. Calcium ion release was

evaluated using Inductively Coupled Plasma (ICP) spectrometry and the pH of the deionized water was recorded via pHmeter. The authors reported and concluded that, Biodentine's pH was modestly and significantly higher from MTA over the entire period. Biodentine maintained alkalinity over the 28 days but the MTA's pH was gradually decreased. Over the entire study period, Biodentine released statistically significantly higher amount of calcium ions than White ProRoot MTA. It was found that, the highest calcium ion release from both materials occurred on Day 1 (195).

Han et al. (2015) tested the calcium release of Endocem MTA, Endocem Zr, and ProRoot MTA materials at periods of 0–5, 6–24, 25–48, and 144–168 hours. Calcium ion levels were evaluated by the ethylene diamine tetra acetic acid (EDTA) titration method. They reported and concluded that, at all measurement points, calcium concentration released from ProRoot MTA was found statistically significantly higher than other materials (196).

Gandolfi et al.(2015) examined CH-based (Dycal, Calxyl red, Calxyl RO blue, Life, Lime-Lite) materials and calcium silicate-based (ProRoot MTA, MTA Plus, MTA Angelus, TheraCal, Biodentine, Tech Biosealer capping) materials for calcium ion release and pH values. The collected sample was analyzed after 3 and 24 hours and 3, 7, 14, and 28 days. pH was evaluated by a selective temperature-compensated electrode, the release of calcium amounts was evaluated by the calcium probe. The cumulative release of calcium was calculated. Although the samples in all material groups released calcium ions, it was observed that the level of calcium release decreased throughout the study period. All calcium silicate-based samples released higher calcium ions than CH-based materials. Higher values for calcium ion release were found for Biodentine, Tech Biosealer capping, MTA Plus gel, and the lowest calcium ion release was found for Lime-Lite. Regarding hydroxyl ion release, it was reported that, all materials released hydroxyl ions with the exception of Life and Lime-Lite. They concluded that high calcium release and pH values of calcium silicate materials might be the reason of the induction of the of new dentin and clinical healing success. (187).

Yamamoto et al. (2017) measured the calcium release and pH levels of TheraCal, a prototype tricalcium silicate cement, and white ProRoot MTA. The periods were 0–5, 6–24, 25–48 and 144–168 hours. Released calcium ions were evaluated by EDTA

titration method and pH was evaluated by pH meter. Within all time intervals, the prototype and MTA's calcium ion release was found significantly greater than TheraCal. The pH of all materials was at peak levels at 5 hours reported to have dropped gradually. At all time intervals, the prototype tricalcium silicate cement and MTA's pH values were reported statistically significantly greater than TheraCal. As conclusion they said that prototype tricalcium silicate cement showed similar calcium ion release and alkalizing activity to ProRoot MTA and these properties of TheraCal were lower than other materials. (197).

Kurun Aksoy et al. (2017) performed an *in vitro* study for comparing the calcium release and pH results of Calcimol, MTA, TheraCal, and Biodentine materials by using 70 freshly extracted third molars with no caries. Calcium release levels were evaluated using an optical emission spectrometer and pH meter was used for hydroxyl ion release. Results were evaluated at 24 hours, 7 days and 28 days. During the study period, all materials released calcium ions. At all periods, Biodentine and TheraCal's calcium release measurements were found higher from other materials but no significant difference was reported between the agents. After 24 hours, Biodentin and TheraCal's pH values were higher than others; nevertheless a decrease in pH was observed towards the end of the study period. They concluded that Biodentine and TheraCal may be the material of choice for indirect pulp capping due to these materials' ion release capacity (198).

Jain et al. (2017) investigated calcium release in forty single-rooted premolars of which roots filled with CH powder mixed with distilled water, Calexcel (CH paste containing propylene glycol) MTA Plus and MTA Angelus. Just the apical third of the roots were immersed in the medium. Calcium ion release of these materials were analyzed using a UV-spectrophotometer at 1, 7, 14, and 28 days. They reported and concluded that initially, MTA-based materials released more calcium ions than CH-based materials. Between the CH agents, Calexcel had the greatest and extended calcium ion release than CH powder mixed with distilled water. Comparing MTA-based products, the highest and stable calcium ion release was reported for MTA Angelus (199).

Pereira et al. (2018); compare the pH values and calcium release levels of Ultra-Blend plus, Biocal (light-cured material) and Hydro C as control group. The samples for Ultra-Blend plus and Biocal were prepared seperately in light-cured and non -cured

forms. The measurements were carried out after 24 hours, 7 and 14 days. pH values were evaluated with digital pHmeter (Q-400 Quimis instrument, São Paulo, SP, BR) and calcium release was evaluated using atomic absorption spectrophotometry (Spectraa 55B - Varian, Inc., Palo Alto, CA, EUA). Highest pH values were found for Hydro C and also calcium release from this material was observed to increase during the test period. For 24 hours and 7 days, when compared to other materials; Ultra-Blend non cured showed more calcium ion release. Also Ultra-Blend non cured's pH and calcium ion release was higher than Ultra-Blend cured. Furthermore, light-cured Biocal released the lowest level of calcium ions and had the lowest pH for all study periods. They concluded that light-cured materials released less calcium ions and presented alteration in pH (200).

Guimarães et al. (2018) evaluated MTA Angelus, MTA Repair HP and MTA Vitalcem for calcium release and pH levels *in vitro* at 3 and 24 hours, and 7, 14, 28 days, using a multiparameter laboratory meter connected to a calcium probe or a (selective) temperature-compensated pH probe/electrode. A pH increase in the medium was observed within the first 7 days for all materials. It was reported that, after 7 days, the materials' alkalinizing activity decreased significantly. MTA Vitalcem's calcium ion release was found substantially greater than other materials after 3 hours. However, from the 1st day to 28th days, the highest and most stable calcium release levels were observed for MTA Repair HP when compared to the other materials (201).

Aprillia et al. (2018) compared the calcium release values of MTA-Angelus and Biodentine *in vitro*. (n=23). They measured the calcium ion release for 1 hour and 49 hours with an atomic absorption spectrophotometer. The authors reported and concluded that at both measurement points the amount of released calcium ions from Biodentine was greater than MTA-Angelus (169).

3. MATERIALS AND METHODS

3.1.Preparation of the Samples

Calcium hydroxide-based materials; Dycal®, Ultra-Blend® plus and calcium silicate based materials MTA Angelus®, Biodentine™ and TheraCal LC® were evaluated in the present study. Table 2 represents the properties (manufacturer, composition, liquid to powder ratio (L/P)) of the materials. (Fig.9)

Fig. 9. Materials used in the evaluation of pH and calcium release

Table 2. Manufacturers, compositions and mixing ratios of the materials.

Material	Composition	L/P ratio
Dycal® (Dentsply, Milford, DE, USA)	*Base Paste:* • 1,3-butylene glycol disalicylate • Zinc oxide • Calcium phosphate • Calcium tungstate • Iron oxide pigments *Catalyst paste*: • Calcium hydroxide • N-ethyl-o/p-toluene sulphonamide • Zinc oxide • Titanium oxide • Zinc stearate • Iron oxide pigments	1 ratio base 1 ratio catalyst
Ultra-Blend® plus (Ultradent Products, Inc, South Jordan, UT)	*Paste:* • Urethane dimethacrylate • Calcium hydroxide	Ready paste
MTA Angelus® (Angelus dental solutions, Londrina, PR, Brazil)	*Powder:* • SiO_2 • K_2O • Al_2O_3 • Na_2O • SO_3 • CaO • Bi_2O_3 • MgO • Insoluble CaO • KSO_4 • $NaSO_4$ • Crystallized silica *Liquid:* • Distilled water	1 scoop powder 1 drop liquid
Biodentine™ (Septodont, France)	*Powder:* • Tricalcium silicate • Dicalcium silicate • Calcium carbonate and oxide • Zirconium oxide (radiopacifier) *Liquid:* • Aqueous calcium chloride solution and excipients • Modified polycarboxylate	1 capsule powder 5 drops liquid

TheraCal LC® (Bisco Inc, Schaumburg, IL, USA)	*Paste:* • Calcium oxide • Calcium silicate particles (type III Portland cement) • Sr glass • Fumed silica • Barium sulphate • Barium zirconate • Resin containing Bis-GMA and PEGDMA	Ready paste

The materials were prepared due to the manufacturers' recommendations. Dycal and MTA Angelus were mixed on a glass plate. Biodentine capsule was mixed with for 30 seconds after 5 drops of liquid added to the capsule. Ultra-Blend plus and TheraCal were light cured after placing into the mold with a light curing unit (1200 mW/cm²) for 20 seconds. The freshly prepared materials were placed into polyvinyl chloride molds which has a diameter: 8.0±0.1 mm and a thickness: 1.6±0.1 mm. 10 samples were prepared for each material (n=10). (Fig.10), (Fig.11).

Fig.10. Polyvinyl chloride molds with a diameter of 8.0±0.1 mm and a thickness of 1.6±0.1 mm

Fig.11. Set materials in polyvinyl chloride molds

Set samples (setting times are for Dycal: 2-3 minutes, for MTA Angelus: 15 minutes, for Biodentine: 10-12 minutes) were immediately immersed in the 10 mL of deionized water in propylene containers and sealed hermetically (Fig.12).

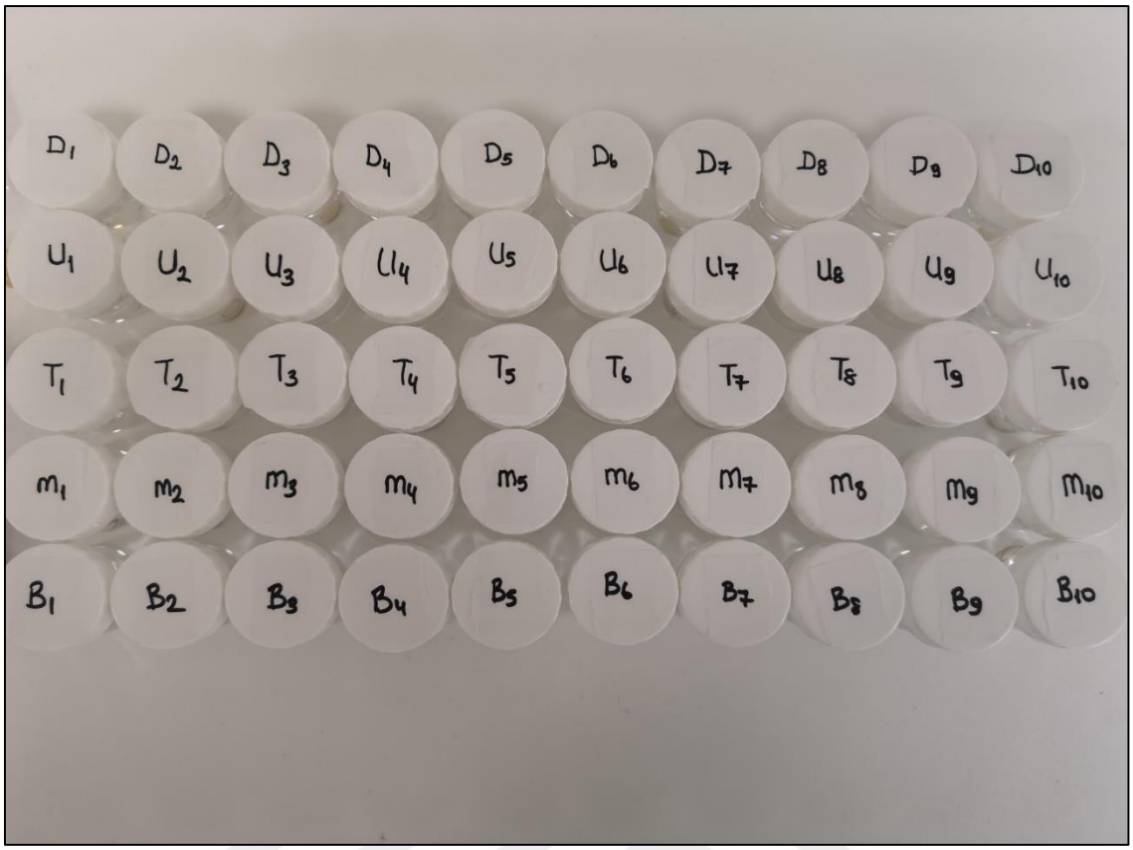


Fig.12. Propylene containers which contain the samples and 10 mL of deionized water

Fig.13. The incubator which provides storage of the samples at 37°C

The samples were stored at 37°C and 100% humidity at incubator throughout the study (Fig.13). At specific measurement days, namely Day 1, Day 3, Day 7, Day 14 and Day 28 the storage media were collected for calcium release and pH evaluation and replaced with the fresh deionized water after each time point.

3.2. Evaluation of pH

The measurements of pH were carried out with a pH-meter (pH 2100 series; pH/mV/Ion/°C/°F Meter with RS232 and Recorder output; OAKTON Instruments, Vernon Hills, IL USA) at Yeditepe University, Faculty of Pharmacy, Analytical Chemistry Laboratory. The calibration of the pH-meter was performed using buffering solutions The electrode of the pH-meter (Mettler Toledo; InLab Expert Pro) was immersed to the sample solutions and maintained until results were stable, and the results were recorded (Fig14.)

Fig 14. pH meter and probe (OAKTON Instruments, Vernon Hills, IL USA)

3.3. Evaluation of Calcium Release

The measurements of calcium release were carried out with the Atomic Absorbtion Spectrophotometer (Zeenit 700 P, Analytik Jena AG, Germany) at Yeditepe University, Faculty of Pharmacy, Pharmaceutical Toxicology Laboratory (Fig.15). Calcium release measurements were performed after pH measurements.

Calcium release was analyzed with an atomic absorption spectrophotometer equipped with a hollow calcium cathode lamp under the following settings: lamp current 3 mA, fuel gas nitrous oxide, support gas oxygen, stoichiometry reducing, wavelength 422.7 nm, and slit width 0.2 nm. A standard stock solution of 10 mg/L calcium was diluted in deionized water to obtain the following concentrations: 0.25, 0.5, 1 ve 2 mg/L. The samples were diluted as necessary (Fig.16). The apparatus was set to zero absorbance using deionized water as blank. A calibration curve was constructed by plotting the concentrations of the standard solutions against absorbances to determine the reliability of the apparatus. After calibration of the apparatus, the water samples in which the materials were immersed were read. Spectrophotometer measured the samples for 3 times and gave a mean value.

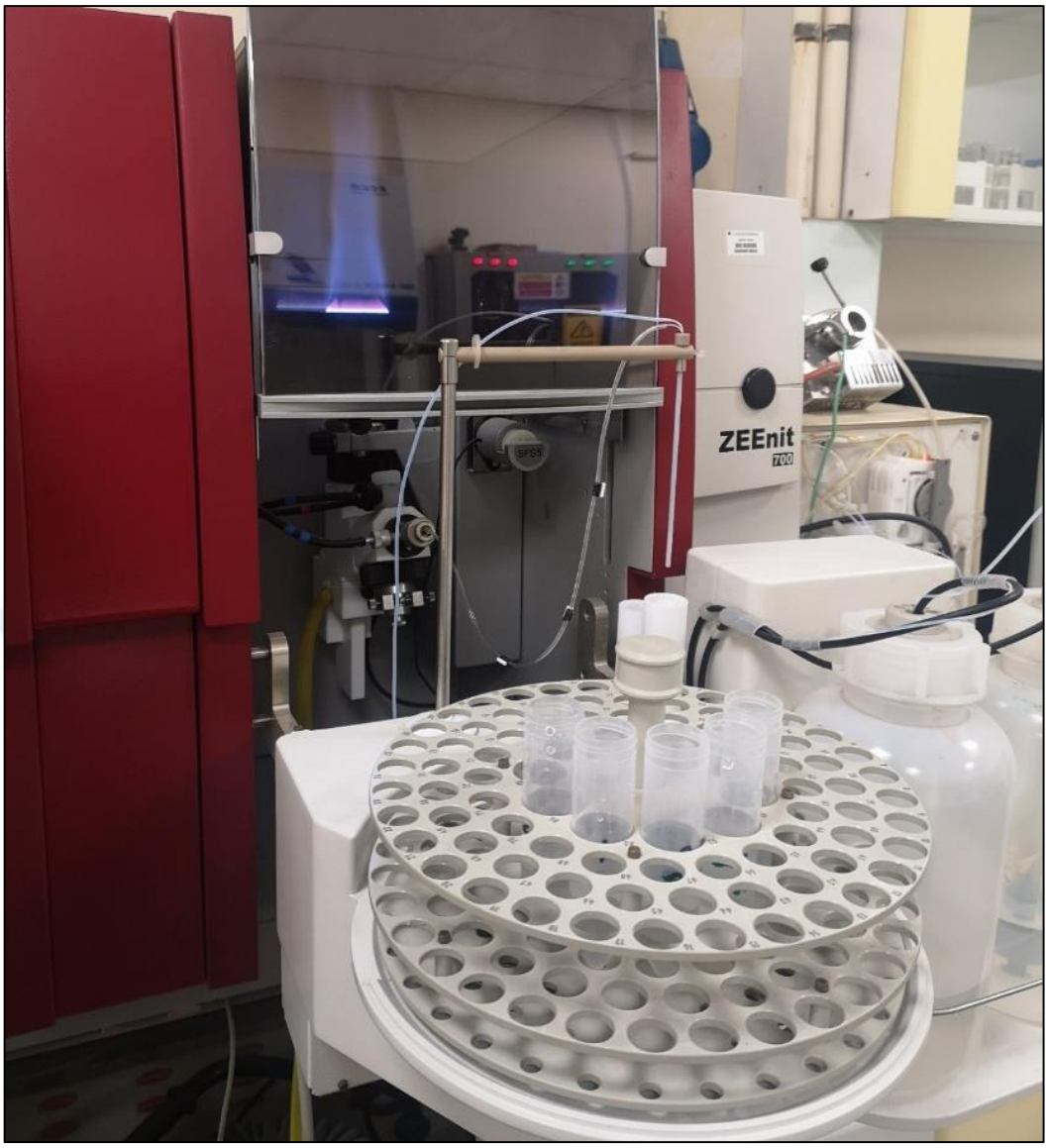


Fig.15. Atomic Absorbtion Spectrophotometer (Zeenit 700 P, Analytik Jena AG, Germany)

Fig.16. Sample solutions evaluated by Atomic Absorbtion Spectrophotometer

3.5. Statistical Analysis

The statistical analysis was carried out using Number Cruncher Statistical System (NCSS) 2007 Statistical Software Program (Utah, USA). Besides descriptive statistical methods (mean, standard deviation), the distribution of variables was examined with the Shapiro - Wilk normality test. One-way analysis of variance was used for the intergroup comparisons of all material groups for different time points, and Tukey multiple comparison test was used for subgroup comparisons. Paired one-way analysis of variance was used in the intragroup comparisons of all time points for every material group, and Newman Keuls multiple comparison test was used in subgroup comparisons, The results were evaluated at the significance level of $p < 0.05$.

4. RESULTS

4.1. Comparison of pH

Table 3 represents the mean and standard deviation of pH values determined in the specific time points for all material groups. Also, comparison of the mean pH values of all material groups with one-way analysis of variance for each measurement day and the comparison of the mean pH values determined in different measurement days with paired one-way analysis of variance for all material groups are presented.

A statistically significant difference was observed between the mean pH values of the material groups at each time point (p = 0.0001). Furthermore, each material group showed statistically significant differences between mean pH values of all measurement days (p = 0.0001) (Table 3).

Table 3: The mean pH values of the material groups measured on different time points.

pH	Dycal	Ultra-Blend plus	Theracal LC	MTA Angelus	Biodentine	p*
Day 1	9.39±0.39	8.98±0.27	9.17±0.28	11.29±0.41	10.32±0.95	**0.0001**
Day 3	9.03±0.13	8.15±0.15	8.48±0.54	10.57±0,66	10.62±0.59	**0.0001**
Day 7	9.23±0.33	8.26±0.41	7.95±0.16	7.85±0.15	7.67±0.14	**0.0001**
Day 14	7.57±0.17	7.29±0.24	7.42±0.12	8.55±1.32	9.00±0.41	**0.0001**
Day 28	9.79±0.76	8.97±0.38	8.61±0.30	8.64±0.37	9.73±0.83	**0.0001**
p‡	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	

***p< 0.05 according to one-way analysis of variance**

‡ p< 0.05 according to paired one-way analysis of variance

The intergroup comparisons between material groups with Tukey's multiple comparison tes*t* for the mean pH values determined on the measurement days are listed in Table 4.

Table 4: The intergroup comparisons of pH values measured on different time points

	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 28**
Dycal / Ultra-Blend plus	0.416	**0.001**	**0.0001**	0.859	**0.021**
Dycal / Theracal LC	0.881	0.086	**0.0001**	0.984	**0.0001**
Dycal / MTA Angelus	**0.0001**	**0.0001**	**0.0001**	**0.01**	**0.0001**
Dycal / Biodentine	**0.002**	**0.0001**	**0.0001**	**0.0001**	0.999
Ultra-Blend plus /Theracal LC	0.926	0.528	0.076	0.991	0.629
Ultra-Blend plus /MTA Angelus	**0.0001**	**0.0001**	**0.008**	**0.001**	0.701
Ultra-Blend plus /Biodentine	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.037**
Theracal LC / MTA Angelus	**0.0001**	**0.0001**	0.911	**0.002**	0.999
Theracal LC / Biodentine	**0.0001**	**0.0001**	0.133	**0.0001**	**0.001**
MTA Angelus / Biodentine	**0.001**	0.999	0.540	0.512	**0.001**

p< 0.05 according to Tukey's multiple comparison test

The mean pH values measured on Day 1 in the MTA Angelus material group was statistically significantly higher than all the other material groups ($p = 0.001$, $p = 0.0001$). Also, the Biodentine material group showed statistically significantly higher pH values compared to the Dycal, Ultra-Blend plus, and Theracal LC material groups ($p = 0.002$, $p = 0.0001$). There were no statistically significant differences between the mean pH values measured in the other material groups on Day 1 ($p> 0.05$).

The mean pH values measured on Day 3 in the Biodentine material group was statistically significantly higher than all the other material groups ($p = 0.0001$). Also, the MTA Angelus material group showed statistically significantly higher pH values compared to the Dycal, Ultra-Blend plus, and Theracal LC material groups ($p = 0.0001$); In addition the Dycal material group also showed statistically significantly higher pH values compared to the Ultra-Blend plus material group ($p = 0.001$), There were no statistically significant differences between the mean pH values measured in the other material groups on Day 3 ($p> 0.05$).

The mean pH values measured on Day 7 in the Dycal material group was statistically significantly higher than all the other material groups ($p = 0.0001$). Also, the Ultra-Blend plus material group showed statistically significantly higher pH values compared to the MTA Angelus and Biodentine material groups ($p = 0.008$, $p = 0.0001$). There were no statistically significant differences between the mean pH values measured in the other material groups on Day 7 ($p > 0.05$).

The mean pH values measured on Day 14 in the Biodentine material group was statistically significantly higher than Dycal, Ultra-Blend plus and Theracal LC material groups ($p = 0.0001$). Also, the MTA Angelus material group showed statistically significantly higher pH values compared to the Dycal, Ultra-Blend plus and Theracal LC material groups ($p = 0.01$, $p = 0.002$, $p = 0.001$). There were no statistically significant differences between the mean pH values measured in the other material groups on Day 14 ($p > 0.05$).

The mean pH values measured on Day 28 in the Dycal material group was statistically significantly higher than MTA Angelus, Ultra-Blend plus and Theracal LC material groups ($p = 0.021$, $p = 0.0001$). Also, the Biodentine material group showed statistically significantly higher pH values compared to the MTA Angelus, Ultra-Blend plus and Theracal LC material groups ($p = 0.037$, $p = 0.001$). There were no statistically significant differences between the mean pH values measured in the other material groups on Day 28 ($p > 0.05$).

The intragroup comparisons of the mean pH values determined in different time points for each material group with Newman–Keuls multiple comparison test are in Table 5.

Table 5: Intragroup comparisons of mean pH values determined in different time points for each material group

	Dycal	Ultra-Blend plus	Theracal LC	MTA Angelus	Biodentine
Day 1 / Day 3	**0.007**	**0.0001**	**0.0001**	**0.005**	0.269
Day 1 / Day 7	0.378	**0.001**	**0.0001**	**0.0001**	**0.0001**
Day 1 / Day 14	**0.0001**	**0.001**	**0.0001**	**0.0001**	**0.003**
Day 1 / Day 28	0.117	0.950	**0.001**	**0.0001**	0.263
Day 3 / Day 7	0.136	0.415	**0.025**	**0.0001**	**0.0001**
Day 3 / Day 14	**0.0001**	**0.0001**	**0.0001**	**0.001**	**0.0001**
Day 3 / Day 28	**0.011**	**0.0001**	0.408	**0.0001**	**0.035**
Day 7 / Day 14	**0.0001**	**0.0001**	**0.0001**	**0.042**	**0.0001**
Day 7 / Day 28	**0.031**	**0.006**	**0.0001**	**0.001**	**0.0001**
Day 14 / Day 28	**0.0001**	**0.0001**	**0.0001**	0.805	**0.027**

‡ p<0.05 according to Newman–Keuls multiple comparison test

In the Dycal material group, the mean pH value determined on Day 14 was statistically significantly lower than the mean values found in other measurement days ($p = 0.0001$). Statistically significantly lower values were found on Day 3 compared to that of Day 1 and Day 28 ($p = 0.011$, $p = 0.007$). The mean pH value on Day 7 was statistically significantly lower than Day 28 ($p = 0.031$); No statistically significant differences were observed between the mean pH values of other time points ($p > 0.05$).

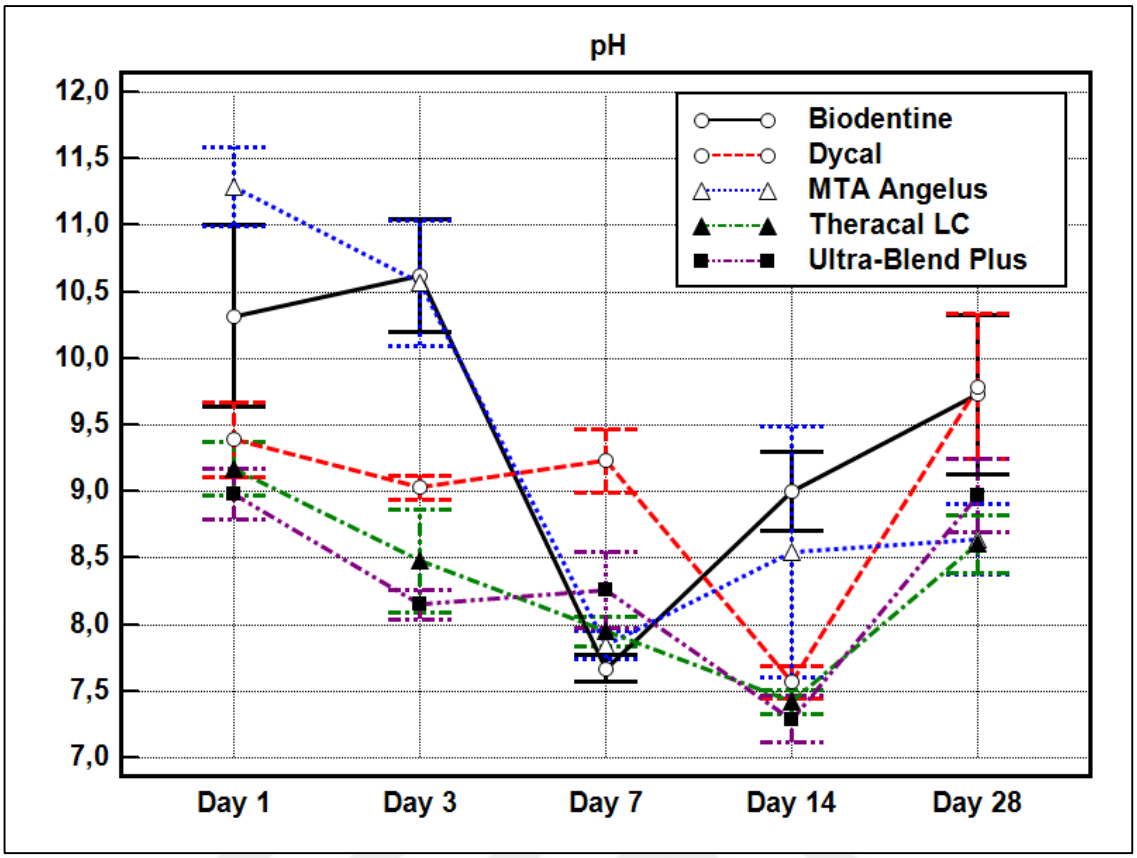
In the Ultra-Blend plus material group, the mean pH value determined on Day 14 was statistically significantly lower than the mean values found in other measurement days ($p = 0.0001$). Statistically significantly higher values were found on Day 1 compared to that of Day 3 and Day 7 ($p = 0.001$, $p = 0.0001$). The mean pH value on Day 28 was statistically significantly higher than Day 3 and Day 7 ($p = 0.006$, $p = 0.001$); No

statistically significant differences were observed between the mean pH values of other time points ($p > 0.05$).

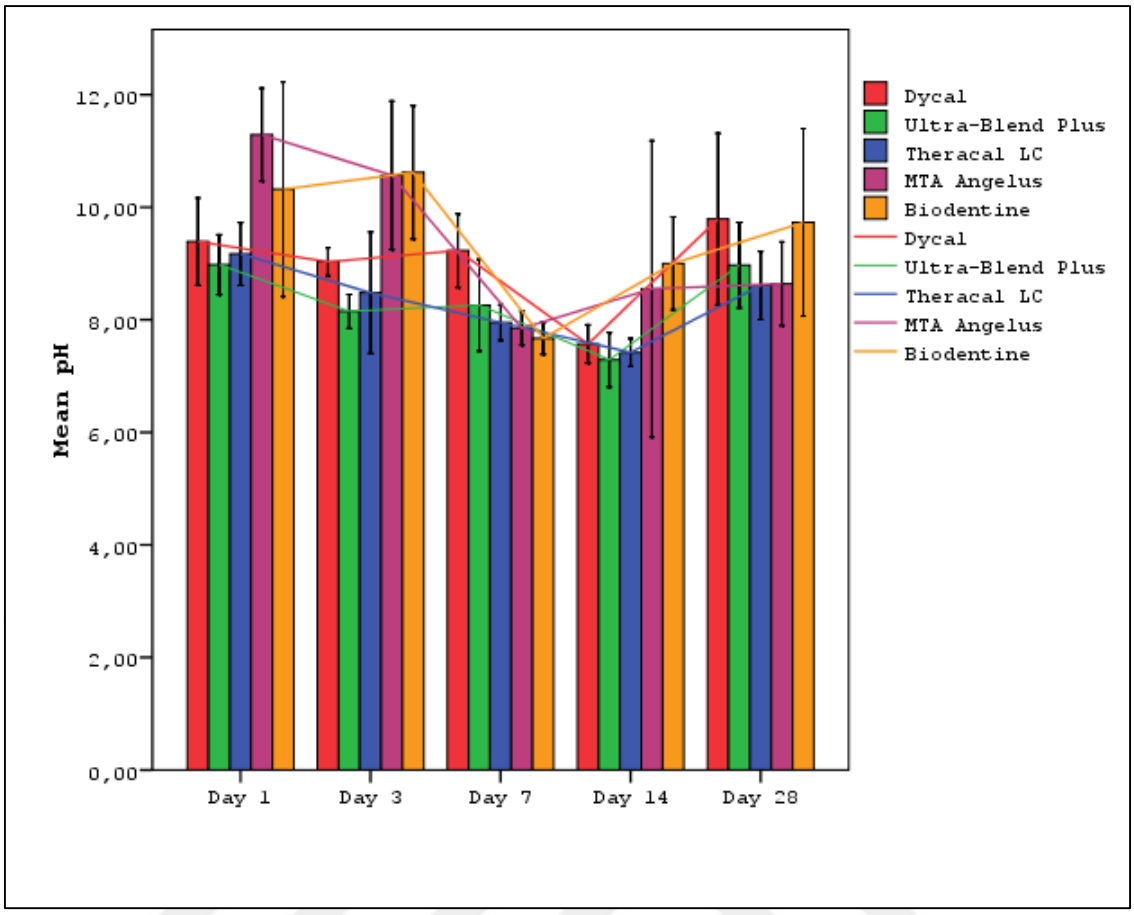
In the TheraCal LC material group, the mean pH value determined on Day 14 was statistically significantly lower than the mean values found in other measurement days ($p = 0.0001$). Statistically significantly lower values were found on Day 7 compared to that of Day 1, Day 3 and Day 28 ($p = 0.0001$). The mean pH value on Day 1 was statistically significantly higher than Day 3 and Day 28 ($p = 0.001$, $p = 0.0001$). No statistically significant differences were observed between the mean pH values of other time points ($p > 0.05$).

In the MTA Angelus material group, the mean pH value determined on Day 1 was statistically significantly higher than the mean values found in other measurement days ($p = 0.005$, $p = 0.0001$). Statistically significantly higher values were found on Day 3 compared to that of Day 7, Day 14 and Day 28 ($p = 0.001$, $p = 0.0001$). The mean pH value on Day 7 was statistically significantly lower than Day 14 and Day 28 ($p = 0.042$, $p = 0.001$); No statistically significant differences were observed between the mean pH values of other time points ($p > 0.05$).

In the Biodentine material group, the mean pH value determined on Day 7 was statistically significantly lower than the mean values found in other measurement days ($p = 0.0001$). Statistically significantly lower values were found on Day 14 compared to that of Day 1, Day 3 and Day 28 ($p = 0.027$, $p = 0.003$, $p = 0.0001$). The mean pH value on Day 28 was statistically significantly lower than Day 3 ($p = 0.027$). No statistically significant differences were observed between the mean pH values of other time points ($p > 0.05$).



Graph 1: The change of the mean pH values during the measurement days for all material groups.



Graph 2: The distribution of the mean pH values in all material groups on different measurement days.

4.2. Comparison of Calcium Release

Table 6 represents the mean and standard deviation of released calcium ions (ppm) determined in the specific time points for all material groups. The comparison of the mean amount of calcium released from the material groups with one-way analysis of variance for each measurement day and the comparison of the mean calcium values determined in different measurement days with paired one-way analysis of variance for all material groups are presented.

A statistically significant difference was observed between the mean amount of calcium released from all material groups at each time point (p = 0.0001). Additionally, each material group showed statistically significant differences between mean calcium values determined for all measurement days (p = 0.0001) (Table 6).

Table 6: The mean and standard deviation of released calcium ions (ppm) determined in the specific time points for all material groups.

Calcium Release	Dycal	Ultra-Blend plus	Theracal LC	MTA Angelus	Biodentine	p*
Day 1	11.79±2.70	27.94±9.43	26.78±10.75	20.11±6.40	97.49±11.39	**0.0001**
Day 3	9.35±2.51	18.65±4.98	32.06±13.01	9.42±2.65	19.80±5.09	**0.0001**
Day 7	16.97±4.58	17.06±5.11	13.28±2.02	12.15±6.32	22.96±2.09	**0.0001**
Day 14	16.00±2.88	8.57±4.63	14.58±2.05	13.36±3.75	19.32±6.14	**0.0001**
Day 28	17.84±5.77	3.74±2.16	13.71±3.16	12.81±4.13	19.49±3.12	**0.0001**
p‡	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	

***p< 0.05 according to one-way analysis of variance**

‡ p< 0.05 according to paired one-way analysis of variance

The intergroup comparisons between material groups with Tukey's multiple comparison test for the mean amount of calcium ions determined on the measurement days are listed in Table 7.

Table 7: The intergroup comparisons of mean calcium release levels measured on different time points

	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 28**
Dycal / Ultra-Blend plus	**0.001**	**0.03**	0.999	**0.002**	**0.0001**
Dycal / Theracal LC	**0.003**	**0.0001**	0.339	0.939	0.137
Dycal / MTA Angelus	0.227	0.999	0.117	0.614	**0.042**
Dycal / Biodentine	**0.0001**	**0.011**	**0.029**	0.39	0.875
Ultra-Blend plus /Theracal LC	0.998	**0.001**	0.315	**0.018**	**0.0001**
Ultra-Blend plus /MTA Angelus	0.282	**0.032**	0.106	0.09	**0.0001**
Ultra-Blend plus /Biodentine	**0.0001**	0.996	**0.032**	**0.0001**	**0.0001**
Theracal LC / MTA Angelus	0.441	**0.0001**	0.978	0.964	0.985
Theracal LC / Biodentine	**0.0001**	**0.002**	**0.0001**	0.095	**0.014**
MTA Angelus / Biodentine	**0.0001**	**0.012**	**0.0001**	**0.019**	**0.003**

$p<0.05$ according to Tukey's multiple comparison test

The mean calcium ion levels measured on Day 1 in the Biodentine material group was statistically significantly higher than all the other material groups ($p = 0.001$). Also, the Dycal material group showed statistically significantly lower calcium levels compared to the Ultra-Blend Plus and Theracal LC material groups ($p = 0.003$, $p = 0.0001$). There were no statistically significant differences between the mean calcium levels measured in the other material groups on Day 1 ($p> 0.05$).

The mean calcium ion levels measured on Day 3 in the Theracal LC material group was statistically significantly higher than all the other material groups ($p = 0.002$, $p = 0.0001$). Also, the Ultra-Blend plus material group showed statistically significantly higher calcium levels compared to the Dycal and MTA Angelus groups ($p = 0.032$, $p = 0.03$). In addition, Biodentine material group showed statistically significantly higher calcium levels compared to Dycal and MTA Angelus groups ($p = 0.012$, $p = 0.011$). There

were no statistically significant differences between the mean calcium levels measured in the other material groups on Day 3 ($p > 0.05$).

The mean calcium ion levels measured on Day 7 in the Biodentine material group was statistically significantly higher than all the other material groups ($p = 0.032$, $p = 0.0001$). There were no statistically significant differences between the mean calcium levels measured in the other material groups on Day 7 ($p > 0.05$).

The mean calcium ion levels measured on Day 14 in the Ultra-Blend plus material group was statistically significantly lower than Dycal, Theracal LC and Biodentine material groups ($p = 0.018$, $p = 0.0001$). Also, the Biodentine material group showed statistically significantly higher calcium levels compared to the MTA Angelus group ($p=0.019$). There were no statistically significant differences between the mean calcium levels measured in the other material groups on Day 14 ($p > 0.05$).

The mean calcium ion levels measured on Day 28 in the Ultra-Blend plus material group was statistically significantly lower than all the other material groups ($p = 0.0001$). Also, the MTA Angelus material group showed statistically significantly lower calcium levels compared to the Dycal and Biodentine material groups ($p = 0.042$, $p = 0.003$). In addition, Theracal LC material group showed statistically significantly lower calcium levels compared to Biodentine group ($p = 0.014$). There were no statistically significant differences between the mean calcium levels measured in the other material groups on Day 28 ($p > 0.05$).

The intragroup comparisons of the mean amount of calcium ions determined in different time points for each material group with Newman–Keuls multiple comparison tes*t* are in Table 8.

Table 8: Intragroup comparisons of mean calcium release levels determined in different time points for each material group.

	Dycal	Ultra-Blend plus	Theracal LC	MTA Angelus	Biodentine
Day 1 / Day 3	**0.004**	**0.001**	0.145	**0.0001**	**0.0001**
Day 1 / Day 7	**0.017**	**0.007**	**0.004**	**0.04**	**0.0001**
Day 1 / Day 14	**0.02**	**0.001**	**0.009**	**0.019**	**0.0001**
Day 1 / Day 28	**0.018**	**0.0001**	**0.007**	**0.005**	**0.0001**
Day 3 / Day 7	**0.0001**	0.384	**0.001**	0.268	0.066
Day 3/ Day 14	**0.0001**	**0.003**	**0.003**	**0.045**	0.874
Day 3 / Day 28	**0.001**	**0.0001**	**0.003**	**0.043**	0.894
Day 7 / Day 14	0.493	**0.001**	0.171	0.526	**0.048**
Day 7 / Day 28	0.640	**0.0001**	0.680	0.769	**0.012**
Day 14 / Day 28	0.215	**0.001**	0.263	0.742	0.932

p<0.05 according to Newman–Keuls multiple comparison test

In Dycal material group, the mean amount of calcium released on Day 3 was statistically significantly lower than the mean calcium release levels on all the other measurement days ($p = 0.004$, $p = 0.0001$). Also, the mean amount of calcium released on Day 1 was found to be statistically significantly lower than that measured on the Day 7, Day 14 and Day 28. ($p = 0.02$, $p = 0.017$). There were no statistically significant differences between the mean calcium release levels of other time points ($p > 0.05$).

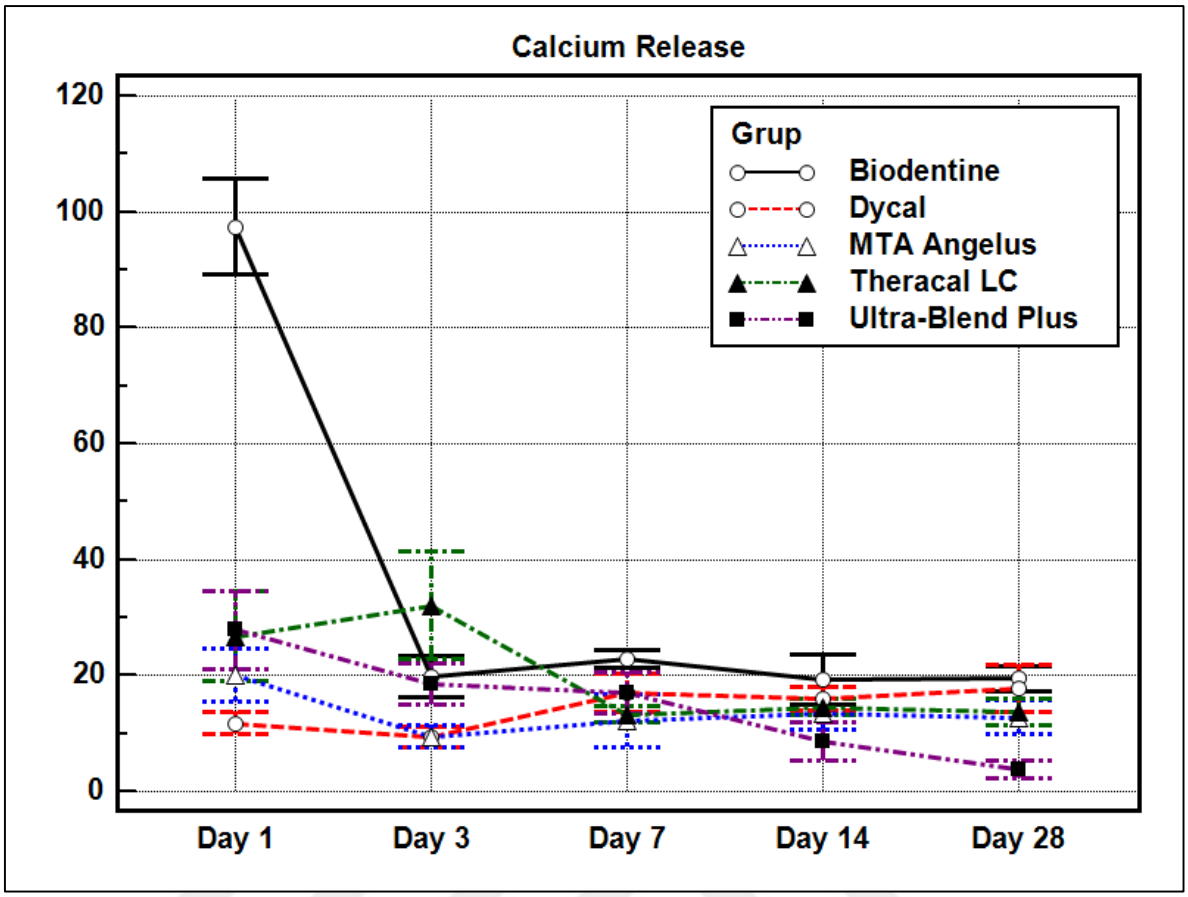
In Ultra-Blend plus material group, the mean amount of calcium released on Day 1 was statistically significantly higher than the mean calcium release levels on all the other measurement days ($p = 0.001$, $p = 0.0001$), Also, the mean amount of calcium released on Day 3 was found to be statistically significantly higher than that measured on the Day 14 and Day 28 ($p = 0.003$, $p = 0.0001$). In addition, the mean amount of calcium released on Day 7 was found to be statistically significantly higher than that measured on the Day 14 and Day 28 ($p = 0.001$, $p = 0.0001$). Finally, the mean amount of calcium released on Day 14 was found to be statistically significantly higher than that measured

on the Day 28 (p = 0.001). There were no statistically significant differences between the mean calcium release levels of other time points (p> 0.05).

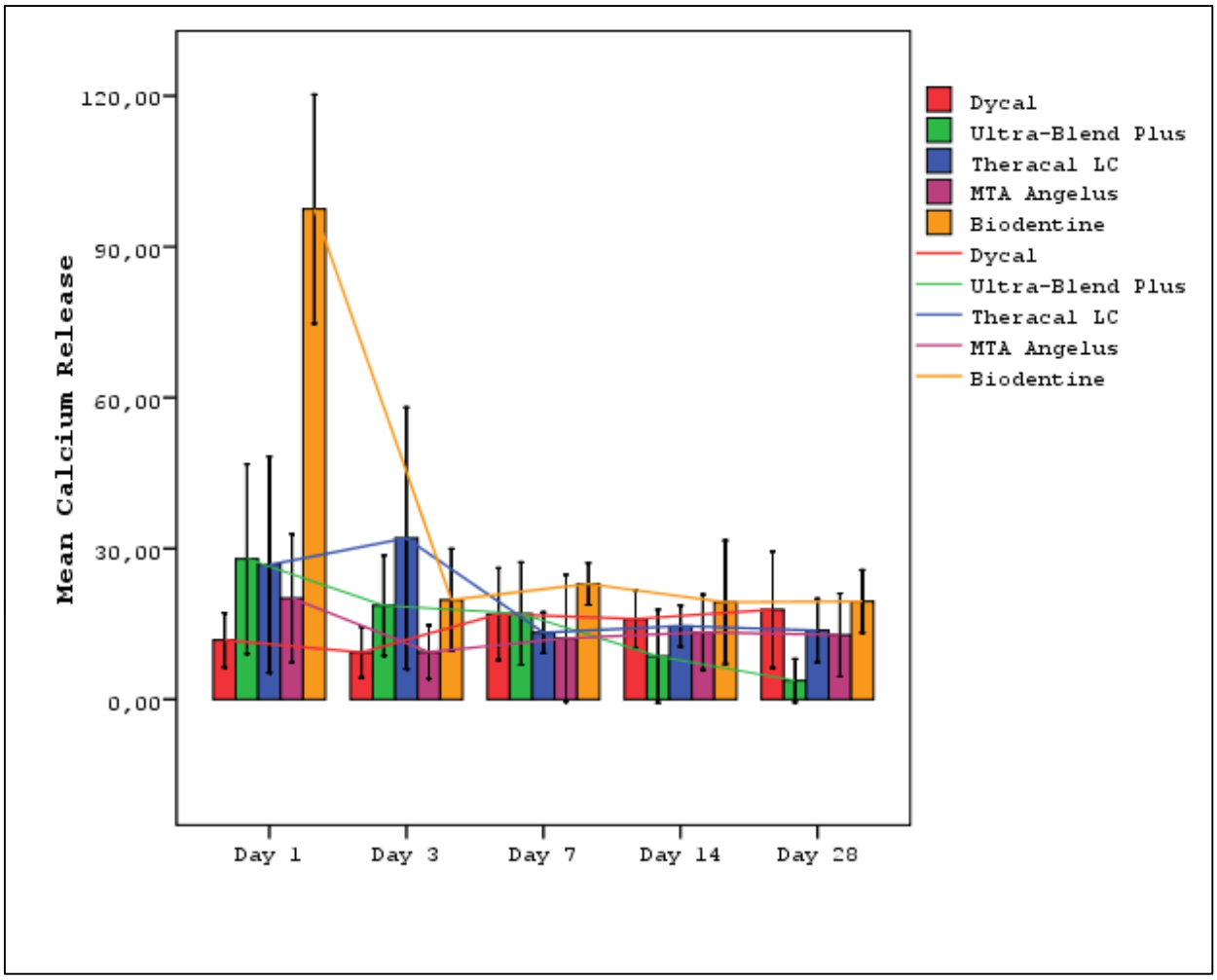
In Theracal LC material group, the mean amount of calcium released on Day 1 was statistically significantly higher than the mean calcium release levels on Day 7, Day 14 and Day 28 (p = 0,009, p = 0,004), Also, the mean amount of calcium released on Day 3 was found to be statistically significantly higher than that measured on the Day 7, Day 14 and Day 28 (p = 0.003, p = 0.001), There were no statistically significant differences between the mean calcium release levels of other time points (p> 0.05).

In MTA Angelus material group, the mean amount of calcium released on Day 1 was statistically significantly higher than the mean calcium release levels on all the other measurement days (p = 0.04, p = 0.0001), Also, the mean amount of calcium released on Day 3 was found to be statistically significantly lower than that measured on the Day 14 and Day 28 (p = 0.045, p = 0.043), There were no statistically significant differences between the mean calcium release levels of other time points (p> 0.05).

In Biodentine material group, the mean amount of calcium released on Day 1 was statistically significantly higher than the mean calcium release levels on all the other measurement days (p = 0.0001), Also, the mean amount of calcium released on Day 7 was found to be statistically significantly higher than that measured on the Day 14 and Day 28 (p = 0.048, p = 0.012). There were no statistically significant differences between the mean calcium release levels of other time points (p> 0.05).



Graph 3: The change in the mean amount of calcium release during the measurement days for all material groups.



Graph 4: The distribution of the mean amount of calcium release in all material groups on different measurement days.

5. DISCUSSION

Clinical practice in dentistry has evolved over the years from the question of how we keep the teeth in the mouth from how we keep the teeth vital in the mouth. While the extraction of teeth was performed instead of the root canal treatment years ago, it is now aimed to keep the tooth vital instead of unnecessary root canal treatments. The reason for this is to ensure the longevity of the tooth and to prevent the disadvantages resulting from root canal treatment. For this reason, vital pulp treatments have gained importance in the recent years. Another important issue, especially in pediatric dentistry, is the state of cooperation. The application of vital therapies that reduce the number of sessions and complications is more important in the treatment of pediatric dental patients. Also, considering that the tooth will be used for many years, the preventive approach gains much more importance in children (8,55).

The purpose of vital pulp therapy is to stimulate the remaining pulp tissue to maintain the vitality of the dental pulp and to regenerate the dentin-pulp complex. Successful vital pulp therapy has two main strategies, to eliminate damage to existing odontoblasts and to allow mesenchymal stem cells to differentiate into new odontoblasts. In the success of the treatment, the degree of pulp damage, the capping material applied, and the effect of bacterial leakage are important factors. The material to be used in the treatment of vital pulp should have the ability to eliminate bacteria, provide mineralization, and create good bacterial hiding. The ideal pulp capping material should be able to resist prolonged bacterial leakage and promote dentin formation by returning the remaining pulp tissue to a healthy state (3,8,69).

In many years, calcium hydroxide-based materials were used for vital pulp therapy; however due to their certain disadvantages, calcium silicate-based materials were produced. Calcium hydroxide and calcium silicate-based materials are stated to have similar mechanisms by releasing calcium and hydroxyl ions to induce hard tissue formation and alkalinize the environment. Calcium silicates form calcium hydroxide with the hydration process and eventually disassociates into calcium and hydroxyl ions (202). The chemical properties of these materials which have been manufactured to be used in vital pulp treatments need to be better understood. In addition to the studies on this subject (169,187,188,203) the calcium and hydroxyl release of the materials containing both calcium hydroxide and calcium silicate were evaluated and compared in the present *in*

vitro study. Thus, in our study, we aimed to provide a better understanding of the properties of these materials and to have more information on this subject by comparing it with previous studies. We believe that, the increase in the number and diversity of *in vitro* studies to be conducted will shed light on *in vivo* and clinical studies and will provide more appropriate and effective usage of materials in clinical practice for vital pulp therapies. Also, these results should be supported by radiologic results in clinical studies in the long term.

Dycal® (Dentsply) is the most known and most used material used for vital pulp therapy as conventional lining material which is introduced in 1962 (187). It is a self-setting (2-3 minutes), two paste system used both in direct and indirect pulp capping procedures. It is widely used for its mineralization stimulating properties, protecting the pulp against thermoelectric stimuli, and supporting the antimicrobial effect (204). Dycal® has a high pH of approximately 12 like other calcium hydroxide liners, which provides excellent antibacterial properties (205). The material hardens with an acid-base reaction and as a result calcium and hydroxyl ions are released. Even though Dycal® has some disadvantages such as high solubility, it is still the gold standard used in the studies aiming to test new materials. In the most previous studies, Dycal® was used as a control group to compare with other materials (9,187,194,203,206).

Ultra-Blend® plus (Ultradent) is a light-cured liner with calcium hydroxide in a urethane dimethacrylate base. Ultra-Blend® plus and these types of resin-based calcium hydroxide materials were produced by adding resin component in calcium hydroxide to overcome the high solubility disadvantage of conventional calcium hydroxides (200). Due to the need for light curing, the working time depends on the clinician and therefore easier to use in clinical practice. Also resin-based calcium hydroxides have better physical characteristics (207). It is shown that un-polymerized resins or monomers are cytotoxic for dental pulp cells (208). Therefore, the use of resin-containing materials in the treatment of direct pulp capping is controversial. In the studies conducted, it was claimed that when the polymerization is done properly and adequately, the resin-modified calcium hydroxide materials are more or less similar to the resin-free calcium hydroxide materials and do not cause a negative response in the pulp cells (209–211). Besides, it was stated that the formation of an oxygen inhibition layer did not reflect the clinical situation sufficiently because these materials were found in the dental interface in a layer lacking

in oxygen (207). For this reason, resin-modified calcium hydroxides are widely used in clinical practice due to their advantages. In our study, we used Ultra-Blend® plus to diversify the calcium hydroxide-based materials.

MTA Angelus® (Angelus) is a calcium silicate-based material which includes Portland type 1 cement. Portland cement materials are composed of dicalcium and tricalcium silicates (140). Silicate materials release calcium and hydroxyl ions like calcium hydroxide as a result of the hydration reaction. MTA was in the crystal phase before the hydration reaction, mainly in this phase as calcium oxide and amorphous calcium phosphate; After the hydration reaction, it has been reported that it hardens and becomes a colloidal gel (135). Calcium silicate-containing materials are tissue-friendly and have high sealing properties. Since they harden with moisture, they stimulate the dentine bridge construction even if they are applied directly on the pulp and have a high clinical success. They are more leak-proof and less soluble than calcium hydroxide-based materials. Also, fewer inflammatory responses, less hyperemia, dentin bridges which are formed faster and thicker have been reported (146,212,213). The inducing effect of MTA on cell proliferation and differentiation is thought to be with the help of calcium ions which are released continuously and regularly from the material (214,215). Calcium and hydroxyl release are also important for apatite forming. Bioactive materials form a calcium phosphate apatite like layer on the surface when faced with a physiological fluid or body fluids. Apatite crystals are formed by the combination of calcium hydroxide, which is formed as a result of hydration of the silicate content, with phosphate ions in the environment. These apatite and calcite crystals formed in the neighborhood of the material serve as the skeleton for the cell attachment, and the dentin bridge is formed around this structure. Besides, it reduces micro-leakage by precipitating between fibrils. Also this layer creates an ideal situation for stem cell differentiation (188). The formation of this layer is attributed to calcium release and maintenance of the high pH for a long time (216). MTA materials have a long setting time. The setting time of ProRoot® MTA was reported as approximately 3 hours in the presence of moisture. Some modifications have made to eliminate this disadvantage. Short setting MTA types are especially important for pediatric dentistry. MTA Angelus® is one of them and set in 12 minutes. This has been achieved by reducing the particle size and adding calcium oxide instead of calcium sulfate (160). It is stated that the high calcium release of MTA Angelus® is due to the high proportion of Portland cement that releases calcium (80%) (217). In our study,

we used MTA Angelus® because it is a favored material in clinical pediatric dentistry practice for its rapid hardening time.

TheraCal LC® (Bisco) is a resin-modified calcium silicate-based material which includes type III Portland cement used in direct and indirect pulp capping treatments (187). Due to its resin content; compared to other conventional silicate materials, it provides fast working time with light polymerization, easy application, good physical properties and these made this material very popular in the recent years and it has been used frequently in many studies (207). TheraCal LC® is claimed to provide adequate dentine stimulation, has strong physical properties, and is therefore successful in pulp therapy (218). In a study conducted for 28 days, it has been shown that more calcium is released from TheraCal LC® than ProRoot® MTA and Dycal®, but it is less dissolved (9). Also it is stated that Ca and OH ions' interaction, released from TheraCal LC® with phosphate ions in biological fluids such as blood, plasma, and dentin fluid, lead to the formation of apatite deposits. This proves the bioactive property of the material (188). Like resin-modified calcium hydroxides, there is a controversy regarding the cytotoxicity levels of TheraCal LC®.The high resin content of the material is thought to pose a risk for pulp viability (219). Some researchers state that most of the monomers cannot react as a result of this high resin content. They stated that the photopolymerization of all monomers is not possible due to the moist environment in direct pulp treatment. These monomers are thought to be cytotoxic to the pulp tissue and fibroblasts; therefore a complete dentine bridge is not formed (220). These and similar studies have said that TheraCal LC® should not be used in direct pulp therapy (218–220). However, the other important point is that TheraCal LC® has a hydrophilic structure compared to resin-based calcium hydroxide materials. This feature causes calcium hydroxide production by hydration reaction like other silicates in the presence of some water absorption. This matrix causes more release of calcium and hydroxide which is necessary for the formation of dentin and antibacterial environment (9,207). In other studies, the material's toxicity or ability to create dentin bridge was found similar to other calcium silicate or calcium hydroxide materials (186,221,222). Although uncured monomers might be toxic to the pulp, in *in vitro* studies it was also indicated that, using resin-based calcium hydroxide or calcium silicate would not be fully compatible with the clinical conditions in terms of the oxygen inhibition layer. Because, in the same way, the material used as a liner between the tooth and the restoration is said to be in the oxygen-free environment (223). Although there are

controversial suggestions on direct pulp capping treatment, we used this material in our study, which has gained attention in the clinical practice in recent years.

Biodentine™ (Septodont) is a tricalcium silicate-based material that is synthetically prepared and presented in a capsulated form. It is produced to eliminate the long setting time of MTA materials. Types of cements with long setting time may be more soluble and have a risk of washout (224). To avoid this problem calcium chloride has been added to the liquid part of the Biodentine™. Calcium chloride in the material quickly penetrates the pores and accelerates hydration. Therefore, the hardening time is reduced. Also, calcium carbonate helps shorten hardening time. Smaller particle size also increases the physical properties and this causes easy handling and a more durable and solid structure (166). Biodentine™ also shows bioactivity by creating an apatite-like surface on the material. These precipitates create a more dense structure and diminish microleakage (197). Being available in a capsule form prevents mixing errors. Moreover, due to its robust structure, its use as a temporary restoration has made it a clinically preferred material. It is produced with active biosilicate technology. In this way, it is possible to create a pure calcium silicate content that enables the removal of undesired products, especially metallic residues (202,225). The setting reaction is similar to other calcium silicates. Calcium silicate gel and calcium hydroxide are formed as a result of hydration with water. This calcium hydroxide breaks down into calcium and hydroxyl ions and causes the start of biological events (202). In a study investigating its use in vital pulp therapies, Biodentine™ was found to be much more successful than calcium hydroxide (CH powder mixed with 0.9% sodium chloride) and ProRoot®MTA in terms of on the effect of stem cells on differentiation, quality of the dentin bridge formed, the minimum level of inflammation and the tolerance of the pulp (226). It has been stated that there is no negative effect on the special functions of mesenchymal cells, it does not have a genotoxic or cytotoxic effect on fibroblasts, and it stimulates the mineralization and formation of a dense dentin bridge by providing differentiation of pulp stem cells to odontoblasts (227). In many studies, Biodentine™ has been shown to release more calcium than calcium hydroxide and other calcium silicate materials in many studies (169,187,188,195,203). In a study conducted in an acidic environment, more calcium release was seen from Biodentine™ than MTA Angelus® and Dycal®. This indicates that when the pulp tissue is inflamed or infected; Biodentine™ is more advantageous where the pH of the environment is low (203). Due to all these positive features, it is a clinically

promising material and it is thought that its use will become more widespread especially in pediatric dentistry. In our study, we used this material to compare with the results of previous Biodentine™ related studies due to these features.

In terms of preparation, there were some differences between the tested materials in the present study. Since Dycal and MTA Angelus are materials which should be mixed manually, unidentical mixing of the samples may have occurred. On the other hand; Biodentine is available in a capsulated form of and mixing with a device reduces this disadvantage and samples are more similar in terms of physical properties than the manually mixed materials. Whereas, Ultra-Blend plus and TheraCal, are light-cured materials, and the important thing is that the material should receive light evenly. This issue was handled in our study by using a light curing device with a tip wider than the diameter of our samples.

In the review of the previous studies, it was observed that there was no certain standard regarding the dimensions of the molds used for the preparation of samples. Molds with different diameters and thicknesses were used in different studies. (169,192,195,201,203,206). In our study, we used diameter and thickness units (diameter:8.0 ±0.1 mm, thickness:1.6±0.1 mm) similar to the studies conducted by ***Gandolfi et al***. (9,187,188,194). The samples were stored in deionized water at neutral pH; to avoid ion contamination from the immersion liquid and standardize the test conditions.

In the previous studies, it was determined that, neutrophilic cell infiltration occurs in about 12 hours, the proliferation of pulpal cells after four days, fibroblasts at the end of seven days, and the formation of irregular hard tissues at the end of three months. It was also stated that, the first repair dentine was seen on the 12th day, the dentin bridge formation was observed on the 21st day and continued for 12 months. Therefore, based on other studies, the duration of the experiment in the present study was determined as 21 days, when the formation of the repair dentin first began to be observed. It is important to achieve continuous release of calcium and hydroxyl ions during this procedure. We evaluated our results as short term (1 day) and long term (21 days) release (228). Since the biological events begin almost within a few hours after the material is placed, ion release must be at an effective level as soon as possible to maintain pulp viability. Therefore, as in other studies, short-term ion release values were examined in

our study. The duration of the experiment was determined as 21 days when the dentine bridge was first seen, like other studies (187,188,192). It was also stated that, the thick dentin barrier formed after 3 months. For this reason, this period should be increased to 3 months follow-up in the future studies (169). In addition, in most studies, cumulative calcium release was not evaluated. Only in limited studies (187,188,195,229), cumulative calcium release was evaluated in addition to evaluations at different time intervals.

In all studies related to pH of dental materials, quantitative pH measurements were made with digital pH meter devices of different brands.

The amount of calcium released from dental materials can be determined via various methods. While calcium probe was used in some studies (9,188,201) the EDTA titration method was preferred in others (196,197,230). The spectroscopic methods are accepted to provide clearer and more accurate measurements. Atomic Absorption Spectrophotometry (169,192,193,200,231) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) are the most used methods in related studies. ***Natale et. al (2015);*** stated that the calcium ratios determined by titration method were lower than ICP-OES. It was suggested that the immediate filtering of suspensions without the opportunity to dissolve large particles was the cause of this difference (203). Atomic Absorption Spectrophotometer (Zeenit 700 P, Analytik Jena AG, Germany) was used for the determination of the amount of calcium release from the test materials in the present study.

Calcium and hydroxyl ion release from the material placed over or close to the pulp tissue is crucial for the success of vital pulp treatment. The ability of releasing these ions is thought to show the biological features of the materials.

Hydroxyl ions help alkaline phosphatase and BMP-2 release which takes part in the calcification process (232,233). Also high pH which is created by the hydroxyl ions causes an antibacterial environment that enhances the success of the treatment Therefore, the ability to release hydroxyl ions is essential for the success of vital pulp therapy (177).

All material groups in our study alkalinized the deionized water throughout the experiment although the pH decreased over time in all groups. This result was similar to the studies conducted which evaluated pH of the pulp capping materials (9,187,188,194,195). Especially on the Day 1, the mean pH of the MTA Angelus and

Biodentine material groups were significantly higher than the other material groups. The hydroxyl release from these two materials decreased to a level close to the values measured in the other material groups. This result is notedly similar to the findings of the study by ***Gandolfi et al. (2015***) which tested almost the same materials groups. It was reported that, MTA Angelus and Biodentine material groups released more hydroxyl ions than the other materials, especially at short term measurements and the pH values decreased over time and became similar to the pH values of other materials, although still slightly higher (187). They also reported similar findings by means of Biodentine material group in the present study, which has still had the higher mean calcium ion release than Ultra-Blend plus, TheraCal and MTA Angelus material groups on Day 28. In our study Dycal, TheraCal and Ultra-Blend plus material groups' pH values generally did not change much during the experiment and maintained their stable pH values with very slight differences. On the Day 28, at the end of the experiment, Biodentine material group was still the material with the highest hydroxyl release together with Dycal material group. Even though the pH of Dycal and Biodentine material groups decreased below 9 at one time point, it remained above 9 for the entire study. This finding is also similar to the values reported by ***Gandolfi et al.*** in that same study. Like our study, the materials with the highest pH values at the end of the experiment were Biodentine and Dycal material groups (187). In other studies comparing Biodentine with ProRoot MTA, the hydroxyl release was found to be higher for Biodentine throughout the whole experiment (188,195). These results have shown that they are suitable materials for hard tissue formation due to the alkaline environment formed.

In the present study, Ultra-Blend plus material group showed alkaline pH throughout the whole experiment. However, in Duarte's study, it was stated that, Ultra-Blend plus material group remained at acidic pH throughout the entire experiment (192). Also in another study; Ultra-Blend plus material group's pH values were determined to be very close to the neutral pH throughout the test period (200). Although the measurement technique is the same, this difference may be due to samples with contact areas in different dimensions in other studies.

Calcium ions are responsible for cell proliferation, differentiation, and consequently hard tissue mineralization (114,186). These ions take part in biomolecule

regulations like osteopontin and growth factors (ex.BMP-2) (118), during the calcification process they also increase pyrophosphatase activity (234).

Continuous release of calcium ions is important for hard tissue formation and maintenance of vitality (9,169). In our study all material groups released calcium ions throughout the test period. Even though all materials released more calcium in short term and the level of calcium decreased over time, calcium release continued at the end of the test period. As an exception, in the Dycal material group an increase in the amount of calcium release was observed from Day 1 and 3 to end of the experiment. Highest calcium ion release was observed on Day 7, 14, 28 and no significant difference was observed between them. It is assumed that, the dissolution of the material in deionized water over time might be the reason for this increase. In other studies, a decrease in the calcium ion release over time was reported for Dycal material group (9,187). In one study release of calcium from Dycal material group increased until 7 days and then decreased after that measurement point (9).

In all the material groups except Dycal and TheraCal, the highest amount of calcium ion release was observed in the first measurement day. Additionally, the Biodentine material group showed the highest amount of calcium ion release among all materials. Although the amount of released calcium ions measured in the Biodentine material group decreased towards the end of the experiment, amount of calcium ion release was higher than other material groups except Dycal material in Day 28. The higher calcium release determined for the Biodentine material group was a finding similar to the results of other studies using the same materials. ***Aprillia*** et al. stated that Biodentine's calcium ion release was found higher than MTA Angelus at 1 hour and at 48 hours (169). Accordingly, ***Gandolfi*** et al. reported that, Biodentine's calcium release was found higher than MTA Angelus, Dycal and TheraCal at 3 hours and 28 days (187). In addition, Biodentine showed higher amounts of calcium release compared to ProRoot MTA in other studies (188,195). The results of the present study are in accordance with those studies that reported higher amounts of calcium release from Biodentine material group, especially in the short term.

Biodentine and MTA Angelus are both tricalcium silicate materials with end products of calcium hydroxide. Also both materials enable crystal deposition which creates hydroxiapatite precipation (169). Despite all these similarities, calcium release of

Biodentine material group was found higher than MTA Angelus material group in short (Day 1) and long term (Day 28) periods in our study and also in other studies (188,195). It was stated that this difference may be attributed to the ingredients of the Biodentine material, such as calcium chloride and calcium carbonate and also low solubility (99,230). Also the small particle size may be responsible for the high ion release. While the diameter of calcium phosphate particles incorporated in Biodentine are smaller than 1 micron, they are 1-5 microns in MTA Angelus. Also, Biodentine's hydration reaction is faster than MTA Angelus. Due to these factors, a more compact surface is formed in Biodentine, that creates lower water absorption and solubility and affects calcium release (169).

In our study more calcium ion relase was found in the TheraCal material group compared to the MTA Angelus and Ultra-Blend plus material groups on Day 3. Furthermore, the TheraCal material group released more calcium ions than the Dycal material group on Day 1 and Day 3. These results were not in accordance with the results reported in the study by ***Gandolfi et al (2015)*** showing MTA Angelus's calcium release was higher than TheraCal in short term period (187). Also in another study ProRoot MTA's calcium release was found higher than TheraCal (197). MTA materials with certain different ingredients produced by various manufacturers may give different results, and comparison of these results may be confounding. Besides, different methods used to evaluate the amount of calcium ion release in the studies may give rise to conflicting results. Calcium probe and EDTA titration methods were chosen in those studies (187,197), whereas atomic absorption spectrophotometer was used in the present study. As we mentioned before, the atomic absorption spectrophotometer method is accepted to give more reliable results (189). Similarly, the TheraCal material group showed no significant difference by means of of calcium release at the end of the experiment compared to the Dycal and MTA Angelus material groups in our study. TheraCal material group was only found significantly higher than Ultra-Blend plus material group on Day 28. In other studies, calcium release of TheraCal was found to be higher than Dycal after 28 days (9,187).

In our study, although the amount of released calcium ions in the Ultra-Blend plus material group was higher than the TheraCal and MTA Angelus material groups on Day 1 and Dycal material group on Day 1 and Day 3, a very rapid decrease on Day 14 and 28 was observed. Ultra-Blend plus material group released the least amount of calcium ions compared to all material groups on Day 28. Prolonged and continuous calcium release from dental materials is a prerequisite for hard tissue formation; nevertheless, there is an enigma for Ultra-Blend plus material. If the duration of the present study was longer than 28 days, would the calcium release diminish by the time? Similar to the results of the present study, ***Pereira et al. (2018)*** reported that, calcium release from Ultra-Blend plus in the short term was higher than conventional calcium hydroxide cement; towards the end of the experiment, they got the opposite result. They mentioned that the reason for this difference might be the setting properties of the material and exposure to light may decrease the amount of calcium release (200). However, this assumption contradicts the high calcium release values of TheraCal material also polymerized via light curing. According to the results of these studies, even if a decrease is observed, the calcium release values are still higher than other conventional calcium hydroxides (9,187). Additionally, TheraCal is a tricalcium silicate-based liner whereas Ultra-Blend plus is a material that consists calcium hydroxide. In another study, Ultra-Blend plus released more calcium than traditional calcium hydroxide cements during the whole experiment (192). However, in the present study although calcium release was higher in the Ultra-Blend plus material group compared to the Dycal material group on Day1 and Day 3 , the amount of calcium ions measured for Ultra-Blend plus were less than Dycal material group at the end of the experiment.

Ion release is related to the network structure, size, density, distribution of the particles of the materials. Water sorption, solubility is affected by these and induce porosity. Because of that in many studies calcium and hydroxyl ion release values are associated with the water sorption and solubility results (169,187,188,194,197,206). ***Chudhari et al. (2016)***; stated that the results of high calcium release in the first few hours of light-cured cements may be the result of low solubility and rapid hydration (206). ***Gandolfi el al. (2014)*** also stated that the rapid hydration reaction of Biodentine can be related to low solubility and high calcium release in the early test period. They also added that these high ion releases can be also related to high open pore volume (187). On the other hand ***Yamamato et al. (2016***) suggested that lower release of ions can be correlated

with lower solubility (197). ***Aprillia et al. (2018)*** stated Biodentine's higher amount of calcium release compared to MTA Angelus might be attributed to the smaller particles of the former (169). ***Rajasekharan et. al. (2018)*** stated in their study that calcium release is increased in an acidic environment, but this situation will cause dissolution from material and affect its physical properties. They also suggested that the surface area and volume of the samples may effect ion release (229).

Limitations of this study

- In order to better understand the ion release, especially calcium release properties, the dental materials should be evaluated in terms of properties such as solubility, water sorption, volume, exposed surface area, acidic and basic environments. In the present study, only pH and calcium ion release properties were investigated. However, water absorption and solubility tests of the same materials are essential for a better interpretation.

- The *in vitro* studies do not exactly reflect the clinical conditions which consist of tissue dynamics, microorganisms, microleakage, and thermal changes in the oral environment. Within the limitations of this *in vitro* study, it has been observed that the tested materials increased the pH and released calcium ions throughout the measurement days. Although these findings might be accepted as a foundation for hard tissue formation, it is not possible to interpret these results and suggest a material of choice for the clinical conditions. These results should be confirmed with randomized prospective clinical studies.

6. CONCLUSIONS

In the present study, the pH and calcium release of Dycal, Ultra-Blend plus, Theracal, MTA Angelus and Biodentine materials currently used in vital pulp treatments were evaluated;

1. All the material groups alkalinized the deionized water throughout the experiment; however the mean pH decreased over time in all material groups.
2. **In short term (Day 1 and Day 3)** significantly **highest** mean **pH** was observed in the **MTA Angelus** and **Biodentine** groups ($p<0.005$).
3. **In long term (Day 28)** significantly **highest** mean **pH** was observed in the **Dycal** and **Biodentine** groups ($p<0.05$).
4. All the material groups released calcium ions throughout the study period and the amount of calcium ions decreased over time in all material groups except Dycal.
5. In the Dycal material group, the mean amount of calcium release tended to increase from Day 3 towards Day 28 and was found significantly higher in Day 7, 14 and 28 when compared to Day 3 ($p<0.005$).
6. **In short term (Day1);** significantly **highest** amount of **calcium** ion release was observed in the **Biodentine** group among all material groups ($p=0.0001$).
7. **In long term (Day 28) Biodentine** group still released more calcium ions than other groups **except Dycal** ($p<0.05$). Significantly **least** amount of **calcium** ion release was found in the **Ultra-Blend plus** material group ($p=0.0001$).
8. **TheraCal** group showed significantly highest amount of calcium ion relase among all groups in Day 3 ($p<0.005$). In Day 28, mean amount of calcium ions in the TheraCal group was only higher than Ultra-Blend plus material group ($p=0.0001$).
9. **MTA Angelus** group showed similar calciumion release with Dycal and Ultrablend plus material groups in Day 1. In Day 28, it was found that, MTA Angelus group's calcium ion release was statistically significantly lower than the Dycal material group ($p<0.05$).

In conclusion; Dycal, Ultra-Blend plus, TheraCal, MTA Angelus, and Biodentine materials used in this study performed hydroxyl and calcium ion release *in vitro* for 28 days, which is required for hard tissue formation. Especially the Biodentine material group significantly had the highest pH and released more calcium ions in short term. However in long term; even though the Biodentine still had the highest pH and amount of calcium ion released; no significant difference was observed between the Biodentine and Dycal material groups. Calcium silicate-based materials, which were produced as an alternative to traditional calcium hydroxide-based ones with better physical properties, have been found successful in the release of ions required for hard tissue formation in this *in vitro* study. Nevertheless, these findings should be supported and confirmed with histopathological and randomized prospective clinical studies.

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