EVALUATION OF NOVEL NANO ZIRCONIA AND NANO GRAPHENE MODIFIED CALCIUM SILICO PHOSPHATE BIOMATERIAL

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EVALUATION OF NOVEL NANO ZIRCONIA AND NANO GRAPHENE MODIFIED CALCIUM SILICO PHOSPHATE BIOMATERIAL

by

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HCS	Hydraulic calcium silicate
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
EDS	Energy dispersive X-ray Spectroscopy
GIC	Glass ionomer cement
CH	Calcium hydroxide
VPT	Vital pulp therapy
MTA	Mineral trioxide aggregate
CPC	Calcium phosphate cement
GIT	Gastrointestinal tract
ATR	Attenuated total reflex
PMMA	Poly methyl methacrylate
IR	Infra-red
mm	Millimeter
g	Gram
mg	Milligram
rpm	Revolution per minute
MPa	Megapascal
F	Load
D	Mean of two diagonals in millimeter
VHN	Vickers microhardness number
KBr	Potassium bromide
UTM	Universal Instron testing machine
IPS	Institut Pengajian Siswazah
USM	Universiti Sains Malaysia

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PANILAIAN NOVEL NANO ZIRKONIA DAN NANO GRAPHENE DIUBAH SUAI BIOMATERIAL KALSIUM SILIKO FOSFAT

ABSTRAK

Matlamat kajian ini adalah untuk menilai sifat mekanikal nano graphene kalsium siliko fosfat dan nano zirkonia kalsium siliko fosfat dengan Biodentine tersedia secara komersial dan menganalisis kimianya menggunakan FTIR dan SEM. Empat kumpulan telah dibuat, ia adalah seperti berikut: kumpulan 1: Biodentine (kumpulan kawalan), kumpulan 2: nano graphene kalsium siliko fosfat (70% Biodentine+20% nano graphene+10% kalsium fosfat), kumpulan 3: nano zirkonia kalsium siliko fosfat (70% Biodentine+20% nano zirkonia+10% kalsium fosfat) dan kumpulan 4: nano zirkonia diubah suai Biodentine (80% Biodentine+20% nano zirkonia). Dua puluh sampel telah disediakan, yang terdiri daripada lima sampel setiap kumpulan. Semua kandungan serbuk bahan pergigian ditimbang pada mesin timbang digital dan dipindahkan ke bekas kedap udara untuk sentrifugasi dan ultrasonik. Cecair kemudiannya ditambah kepada kandungan serbuk mengikut arahan pengilang dan amalgamator digunakan untuk mencampurkan serbuk dan cecair. Campuran itu kemudiannya dipindahkan ke dalam acuan akrilik berbentuk cakera dan dibiarkan pada suhu bilik semalaman diikuti dengan meletakkan sampel dalam inkubator selama 24 jam pada suhu 37ºC dan kelembapan 100%. Selepas set awal semua kumpulan, sampel dirobohkan dan dihancurkan dengan alu dan mortar menjadi serbuk halus. Sampel hancur digunakan untuk analisis FTIR dan SEM EDX. Penguji microhardness Vickers digunakan untuk mengukur kekerasan mikro dan mesin ujian Instron universal digunakan untuk memeriksa kekuatan mampatan sampel. Analisis data dilakukan enggunakan ujian ANOVA Sehala dan ujian post-hoc Games-Howell. Aras keertian ialah $p<0.05$. Telah diperhatikan dalam spektrum Biodentin dan kumpulan diubah suainya 2,3, dan 4 bahawa getaran regangan beralih daripada 3461.10 cm-1 kepada 470.84 cm-1. Analisis SEM EDX menunjukkan taburan zarah serpihan yang sekata (1-10 µm) dalam berganding dengan bulat (1 - 10 µm) dan dengan zarah yang lebih kecil daripada 0.1 µm diperhatikan. Kumpulan 3 (nano zirconia calcium silico phosphate) dan kumpulan 4 (nano zirconia modified Biodentine) mempunyai kekerasan mikro yang lebih tinggi daripada kumpulan 1 (Biodentine). Nilai mikrohardness paling sedikit direkodkan oleh kumpulan 2 (nano graphene calcium silico phosphate). Kekuatan mampatan tertinggi diperhatikan oleh kumpulan 3 (nano zirkonia kalsium siliko fosfat) diikuti oleh kumpulan 1 (Biodentine) dan kumpulan 2 (nano graphene kalsium siliko fosfat) dan nilai terkecil direkodkan oleh kumpulan 4. Biodentine ialah bahan biomimetik inovatif yang digunakan dalam pelbagai bidang pergigian kerana sifat biologinya yang sesuai dan ciri fizikal dan mekanikal yang lebih tinggi seperti dentin semula jadi. Nano zirkonia kalsium siliko fosfat kumpulan dalam kajian ini menunjukkan keputusan yang menggalakkan. Terdapat peningkatan ketara dalam kekerasan mikro dan kekuatan mampatan dengan penambahan nanozirkonia dan trikalsium fosfat kepada Biodentine. Kajian lanjut diperlukan untuk menilai ketoksikan, biokompatibiliti dan sifat osteokonduktif bahan baru.

EVALUATION OF NOVEL NANO ZIRCONIA AND NANO GRAPHENE MODIFIED CALCIUM SILICO PHOSPHATE BIOMATERIAL

ABSTRACT

The aim of this study was to evaluate the mechanical properties of nano graphene calcium silico phosphate and nano zirconia calcium silico phosphate with commercially available Biodentine and analyze their chemistry using FTIR and SEM. Four groups were made, they are as follows: group 1: Biodentine (control group), group 2: nano graphene calcium silico phosphate (70% Biodentine+20% nano graphene+10% calcium phosphate), group 3: nano zirconia calcium silico phosphate (70% Biodentine+20% nano zirconia+10% calcium phosphate) and group 4: nano zirconia modified Biodentine (80% Biodentine+20% nano zirconia). Twenty samples were prepared, which consist of five samples of each group. All the powder contents of dental material were weighted on a digital weight machine and transferred to an airtight container for centrifugation and ultrasonication. Liquid was then added to powder content as per the manufacturer's instruction and amalgamator was used to mix the powder and liquid. The mixture was then transferred to disk shaped acrylic molds and allowed to set at room temperature overnight followed by placing the samples in an incubator for 24 hours at 37ºC and 100% humidity. After the initial set of all the groups, samples were demoulded and crushed with pestle and mortar into fine powder. Crushed samples were used for FTIR and SEM EDX analysis. The Vickers microhardness tester was used to measure the microhardness and universal Instron testing machine was used to check the compressive strength of samples. Data analysis was done using One-way ANOVA test and Games-Howell post-hoc test.

Significance level was $p<0.05$. It was noted in the spectra of Biodentin and its modified groups 2,3, and 4 that the stretching vibration shifted from 3461.10 cm^{-1} to 470.84 cm^{-1}.SEM EDX analysis shows even distribution of splintered particles (1 - 10 µm) in conjunction with round (1 - 10 µm) and with particles smaller than 0.1 µm were observed. Group 3 (nano zirconia calcium silico phosphate) and group 4 (nano zirconia modified Biodentine) had higher microhardness than group 1 (Biodentine). Least microhardness value was recorded by group 2 (nano graphene calcium silico phosphate). Highest compressive strength was noticed by group 3 (nano zirconia calcium silico phosphate) followed by group 1 (Biodentine) and group 2 (nano graphene calcium silico phosphate) and the least value was recorded by group 4. Biodentine is an innovative biomimetic material applied in various fields of dentistry due to its suitable biological properties and higher physical and mechanical characteristics like natural dentine. Nano zirconia calcium silico phosphate group in this study showed favorable results. There was a noticeable increase in microhardness and compressive strength with the addition of nanozirconia and tricalcium phosphate to Biodentine. Further research is required to evaluate the toxicity, biocompatibility and osteoconductive nature of novel material.

CHAPTER 1

INTRODUCTION

1.1 Background

Dental cement binds the restoration and prepared tooth; it increases the resistance against dislodgement and maintains the seal between the two (Jankar *et al.*, 2021). Hence, for a successful restoration selection of appropriate cement is crucial(Simon and de Rijk, 2006). Various dental cements have been introduced in dentistry over the decades, and hydraulic calcium silicate-based cements (HCS) are one of them. They have gained popularity since their introduction due to their capacity to remineralize or regenerate dental tissues (Prati and Gandolfi, 2015).

Although numerous HCS cements are accessible however, Biodentine has been emphasized from the time of its introduction in 2009 for widespread application in dental procedures such as apexification, perforation, root repair, pulp capping, pulpotomy, internal and external root resorption, and retrograde filling (Malkondu *et al.*, 2014). They are used in deep coronal caries, cervical lesions, and endodontic surgeries (Jefferies, 2014). Powder of Biodentine is composed of tricalcium silicate (main core), dicalcium silicate (second core), zirconium oxide (radio pacifier), calcium carbonate (filler), and other oxide fillers. The liquid contains calcium chloride (accelerator), hydro-soluble polymer (water-reducing agent), and water (Camilleri, 2013; Malkondu *et al.*, 2014). It is an efficient 'dentine substitute' with the shortest setting time, lower porosity, efficient sealing ability, color stability, and good physio-mechanical properties (Rajasekharan *et al.*, 2018a). It is biocompatible and antimicrobial, capable of inducing dentin mineralization and promoting the formation of tertiary dentin (Tomás-Catalá *et al.*, 2018).

Brown and Chow introduced calcium phosphate cement in 1980 (WE, 1983). It is a promising material in dentistry because of its advantageous features like biocompatibility, bioactivity, osteoconductivity, and moldability (Ginebra *et al.*, 2012). They are self-setting cements capable of hardening in vivo at low temperatures and have the potential to bond directly with bone (Ambard and Mueninghoff, 2006). However, mechanical properties are the primary concern as they are porous and brittle with less tensile strength and impact resistance (Ambard, 2001; Ambard and Mueninghoff, 2006).

Nanotechnology is another emerging field in dentistry where nanoparticles enhance the physio-mechanical properties of dental materials (Agnihotri *et al.*, 2020). Nano zirconia is a nanomaterial with light particles; it is biocompatible and has advantageous mechanical characteristics like higher hardness, fracture toughness, and flexural strength (Bapat *et al.*, 2022). It is an aesthetic with good white color, steady chemical features, and increased resistance to corrosion. Hence, they are beneficially used to enhance the mechanical properties of dental materials (Agnihotri *et al.*, 2020; Alhavaz *et al.*, 2017). Another promising nanomaterial is graphene, introduced in 2004 (Novoselov *et al.*, 2004). It is a carbon-based two-dimensional structure where atoms are organized in a honeycomb hexagonal pattern with a large surface area and remarkable mechanical, thermal, and electrical properties (Radhi *et al.*, 2021). It is bio-compatible and has antibacterial effects against gram-positive and gram-negative bacteria. Nano graphene is used in various fields of dentistry, such as implants and fillers in dental cement, and it is popularly used in tissue engineering (Tahriri *et al.*, 2019).

Microhardness of a material is defined as the resistance or strength of the material to deformation (Batul *et al.*, 2023). It is not the measurement of individual property but is influenced by additional fundamental characteristics of the material like modulus of elasticity, tensile strength, and crystal structure stability (Poplai *et al.*, 2013). It is measured by marking the indentations on the surface of a material (Batul *et al.*, 2023). Compressive strength is the tolerance of a material to the excessive vertical force applied before fracture. This is an important material mechanical property tested using a Universal Instron testing machine (Sheykhrezae *et al.*, 2018).

Characterization of material can be known by Fourier transform infrared spectroscopy (FTIR), the vibrational technique widely used for quantitative and qualitative analysis of samples, further to know the organic and inorganic composition of the material (Assiry *et al.*, 2023). Scanning electron microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDS) is other techniques that can potentially provide fundamental information about surface topography, chemical composition, and crystalline structure of organic and inorganic materials on a nanometer level (Mohammed and Abdullah, 2018; Vernon-Parry, 2000).

1.2 Problem Statement

The discovery of ideal cement is not yet complete, although it must have specific criteria like biocompatibility, enough working time, sealing ability, being harmless to tooth and surrounding structures, good mechanical properties and strength, microleakage, and intact restoration (Wingo, 2018). The choice of dental material depends on the tooth condition; amalgam and Glass-ionomer cement (GIC) are usually used to restore the posterior teeth with deep carious lesions (Koubi *et al.*, 2013). However, amalgam use is declining due to its poor aesthetics and mercury toxicity

(Bates, 2006). Subsequently, fluoride release and molecular adhesion to teeth are the main advantages of Glass-ionomer cement (Wingo, 2018), but there is no evidence of dentine bridge formation or stimulation of reparative dentin (Kadali *et al.*, 2021).

The selection of material changes when the pulp is involved, as maintaining the pulpal vitality is crucial for the tooth's survival (Arandi and Thabet, 2021). To stimulate the tertiary dentin formation and retain the functionality of the tooth, Vital pulp therapy (VPT) is performed, which varies from conservative procedures like indirect and direct pulp capping to invasive treatments such as pulpectomy and pulpotomy (Ghoddusi *et al.*, 2014). Calcium hydroxide was considered the gold standard material for direct pulp capping since it has an antimicrobial effect and can stimulate the formation of tertiary dentin (Arandi, 2017). Despite that, it dissolves over time and has shown tunnel defects underneath the dentin bridge, poor sealing, and low compressive strength (Hilton, 2009). Later, tricalcium silicate cements were introduced, which mainly included mineral trioxide aggregate (MTA), ProRoot MTA, TheraCal, Bioaggregate, Endosequence root repair material and Biodentine (Septodont, Saint-Maur-des-Fossès, France) that appeared to be the absolute alternative for direct pulp capping (Cushley *et al.*, 2021).

MTA, introduced in 1993, is a hydrophilic and biocompatible cement that can be applied in conservative and endodontic procedures (Cervino *et al.*, 2020). MTA can stimulate the cell proliferation and differentiation of hard tissues when it comes in contact with the dental tissues during placement. However, tooth discoloration, long setting time, high cost, and difficulty handling are possible drawbacks to introducing another bioactive material Biodentine (Parirokh *et al.*, 2018).

Biodentine is a notable representative of calcium silicate-based materials, and it was introduced in 2011. It is biocompatible, antimicrobial, radiopaque cement with the advantage of the shortest setting time, does not cause tooth discoloration and has favourable mechanical properties (Batul *et al.*, 2023). Vickers microhardness is an important property of material which can be described as resistance to plastic deformation of material after indentation. Microhardness of Biodentine is almost similar to natural dentin and hence its mechanical behaviour is identical to human dentin. Therefore, it can be used as dentin substitute {Kaup, 2015 #3}. Compressive strength displays strength of the material. Compressive strength of Biodentine is close to human dentin, the smooth surface of Biodentine that consist of fine particles which adheres to one another and continuation of crystallization up to four weeks may be attributed to increase strength of the material {Butt, 2014 #7}. However, its main drawbacks are low radiopacity, poor bonding strength, and less washout resistance (Kaur *et al.*, 2017). A German, French, and Norwegian study revealed that Biodentine was less common among dental practitioner procedures like direct pulp capping and partial pulpotomy. Calcium hydroxide was preferred over Biodentine; the primary reason for this preference was deficient training and cost (Arandi and Thabet, 2021). Another drawback is that it requires layering in deep caries; after validating the health of the pulp, Biodentine is partially removed, followed by the placement of another permanent dental cement (Koubi *et al.*, 2013).

Therefore, this study was conducted to obtain a new biomaterial by modifying the Biodentine with calcium phosphate and nanomaterials (nano zirconia and nano-graphene) to enhance the mechanical properties of Biodentine and ease the dental procedure by minimizing the usage of the number of dental materials.

1.3 Justification

The proposed study aims to enhance the mechanical properties of Biodentine by modifying it with materials like calcium phosphate and nanomaterials (nano zirconia and nano-graphene) in definite proportions. This study will help the practitioner to compare the mechanical properties of Biodentin with newly modified biomaterial and assist dentists in using it in dental procedures like direct pulp capping as permanent biomimetic material.

The materials used in the study to modify Biodentine are justifiable. Calcium phosphate materials are biomedical; they are biocompatible and non-toxic in nature and are attentively used in medical and dental fields owing to their similar chemistry to teeth and bone (Al-Sanabani *et al.*, 2013). Calcium phosphate cement (CPC) was introduced by Brown and Chow, a self-hardening cement that indicates the repair and differentiation of living tissues by forming a positive interaction with them. It has antimicrobial properties; thus, all these characteristics allow this material to be potentially used for regenerating dentin in pulp capping (Al-Sanabani *et al.*, 2013; Shieh *et al.*, 2017).

They are synthetic bioactive materials, classified as hydroxyapatite and tricalcium phosphate based on resorbability; further, they have noticeable features like osteoconductivity and osteointegrativity (Ginebra *et al.*, 2012). They are extensively used in bone defects, bone implants, as scaffolds, and in various orthopaedic applications besides drug delivery materials (Ginebra *et al.*, 2012; Schröter *et al.*, 2020; Xu *et al.*, 2017). When combined with CPC, tricalcium silicate cements are considered endodontic materials used in numerous dental procedures (Shieh *et al.*, 2017). Due to hydroxyapatite's bioactive and osteoconductive features, it is well preferred for forming reparative dentin, coating dental implants, and treating periodontal diseases (Al-Sanabani *et al.*, 2013).

Nanotechnology is a promising field in dentistry that deals with particles of nanometre size, called nanoparticles, that can enhance material properties (Priyadarsini *et al.*, 2018). Nano-structured materials undergo catalytic and oxidative reactions, and if these reactions induce cytotoxicity, then toxicity could be greater than that of identical material in bulk. The exposure of nanoparticles-reinforced dental materials is through the gastrointestinal tract (GIT); Zirconia has low water solubility, and its absorption in GIT is negligible and is considered a low-toxicity material (Alhavaz *et al.*, 2017).

Zirconia is a biomaterial with white crystalline oxide of zirconium; it is biocompatible, highly aesthetic, and radiopaque with stable and superior chemical and mechanical properties, increasing corrosion resistance, hence extending its application in dentistry. It is evident from the properties mentioned above that incorporating zirconia nanoparticles into different materials significantly enhances their properties (Bapat *et al.*, 2022). This can be explained by the studies where nano zirconia added groups of polymethyl methacrylate denture base exhibited a significant increase in the surface hardness, impact strength, and flexural strength compared to un-reinforced groups (Ergun *et al.*, 2018; Gad *et al.*, 2016b). Accordingly, reinforcement of nano zirconia to GIC increased its surface microhardness, flexural strength, and compressive strength. Thus, the overall enhancement of mechanical properties (Alobiedy *et al.*, 2019; Melo *et al.*, 2019).

The other nanofiller used in the study is graphene. Graphene is the mother of all graphitic forms of carbon, and it is the most robust known material. It is harder than diamond; however, it has a greater surface area, the highest Young's modulus, and fracture toughness. Despite its hardness, it is more elastic than rubber.(Papageorgiou *et al.*, 2017). It is already used in dental implants and removable prostheses, and its use in endodontic dentistry would also be of great importance.

1.4 General Objective

The study aims to develop new biomaterials by adding nanoparticles like nano zirconia and nano-graphene to Biodentine along with calcium phosphate and evaluate the mechanical and chemical properties of these newly formulated biomaterials.

1.4.1 Specific Objective 1

To compare the chemistry (FTIR) of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine.

1.4.2 Specific Objective 2

To compare the SEM and EDS analysis of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine.

1.4.3 Specific Objective 3

To compare the microhardness of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine.

1.4.4 Specific Objective 4

To compare the compressive strength of newly formulated nano graphene calcium silico phosphate nano zirconia calcium silico phosphate, and nano zirconia modified Biodentine with commercially available Biodentine.

1.5 Research Questions

1. Is there a significant variation in the chemistry (FTIR) of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate, and nano zirconia modified Biodentine with commercially available Biodentine?
2. Is there a significant variation in the SEM and EDS analysis of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate, and nano zirconia modified Biodentine with commercially available Biodentine?
3. Is there a significant variation in the microhardness of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine?
4. Is there a significant variation in the compressive strength of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine?

1.6 Null Hypothesis

1. There is no significant variation in the chemistry (FTIR) of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine?
2. There is no significant variation in the SEM and EDS analysis of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium

silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine?

3. There is no significant variation in the microhardness of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine?
4. There is no significant variation in the compressive strength of newly formulated nano graphene calcium silico phosphate, nano zirconia calcium silico phosphate and nano zirconia modified Biodentine with commercially available Biodentine?

CHAPTER 2

LITERATURE REVIEW

2.1 Calcium Silicate Cement

Calcium silicate-based cements are self-setting cements. Their biocompatibility, sealing ability and physiochemical properties are the primary factors which make them suitable to be used in various clinical conditions like pulp capping, pulpotomy, perforation, apexification, apexogenesis and root-end filling (Dawood *et al.*, 2017).

Biomimetics is another field that can be described as the study of biologically produced materials' formation, structure, and function, mainly for synthesizing products that imitate natural ones by artificial mechanisms. Thus, the material formed by the biomimetic process is known as the biomimetic material (Kottoor, 2013). The term "Bio" in Greek stands for life, and "mimesis" for imitate.

Biomimetic dentistry is an interdisciplinary field that replaces lost dental tissues with a restorative material to restore the tooth's full function, strength, and aesthetics (Srinivasan and Chitra, 2015). Conventional procedures involve the removal of more tooth structures, followed by their replacement with rigid materials. However, these materials and methods weaken the tooth structure and reduce restoration longevity. Hence, it is essential to use materials that can regenerate and replace lost dental structures through procedures that imitate natural ones (Goswami, 2018).

Over the decades, various biomimetic materials have been introduced in dentistry to repair the affected tooth, like calcium hydroxide, glass ionomer cement (GIC), dental composites, ceramics, bioglass, and calcium silicate-based cements like MTA, Biodentine, bio aggregate, TheraCal, Endosequence root repair material and calcium-enriched mixture cement (Singer *et al.*, 2023). These restorative materials must be biocompatible, retain natural tooth functions, maintain pulpal vitality, stimulate reparative dentin formation, prevent microleakage and bacterial invasion, bond with the tooth to provide near-normal biology, and be aesthetic (Qureshi and Soujanya, 2014; Singer *et al.*, 2023).

2.2 Calcium Hydroxide

Herman introduced calcium hydroxide in 1920 as a pulp-capping agent that has been used in dentistry for over a century in various fields of endodontics. It is a white powder and is chemically strong. When it comes in contact with an aqueous solution, it separates into calcium and hydroxyl ions(Ba-Hattab *et al.*, 2016). It is popularly used in dentistry due to various properties like remineralization, antibacterial effect, dentin bridge formation, and necrotic material dissolution (Reddy *et al.*, 2020).

2.2.1 Advantages and Disadvantages of Calcium Hydroxide

Calcium hydroxide has been regarded as the 'gold standard' of direct pulp capping for other restorative materials over the decades due to its antimicrobial properties, which can reduce or eliminate bacterial invasion. Its success rate can be tracked over the years, but a few apparent drawbacks must be considered. The advantages and disadvantages of calcium hydroxide are listed in Table 2.1.

Table 2.1 Advantages and disadvantages of calcium hydroxide (Ba-Hattab *et al.*, 2016; Lin *et al.*, 2016)

Advantages of calcium hydroxide	**Disadvantages of calcium hydroxide**
Antibacterial property	High solubility
Promotes repair and healing	Solely do not stimulate dentin bridge or reparative dentin formation.
Induces mineralization	Primarily related to tooth resorption
High Ph causes stimulation of fibroblast.	Dissolve over a period and cause tunnel defects in reparative dentin.
Low cost and easy to use	Higher chances of tooth fracture
	Increased and variable treatment time makes it difficult to follow up with the patients.
	Do not adhere to dentin or restoration.

2.2.2 Clinical Usage of Calcium Hydroxide

Calcium hydroxide is applied in various endodontic procedures due to its low cytotoxicity, antibacterial properties, distinctive mechanism of action, and long-term record in dentistry (Reddy *et al.*, 2020). Applications of calcium hydroxide is listed in Table 2.2 below.

Table 2.2 Application of calcium hydroxide (Ba-Hattab *et al.*, 2016; Reddy *et al.*, 2020)

Application of calcium hydroxide
Popularly used as an intercanal medicament.
Used as cavity liner
As a pulp capping agent
As temporary material in apexification
In pulpotomy
As root canal sealer
In root resorption and periapical lesion

Although calcium hydroxide is considered the gold standard of direct pulp capping and has a long history of use in dentistry, various studies are reporting its failure high failure rate due to limitations like microleakage, tunnel defect formation, degradation over time, and the presence of inflammation in treated teeth (Li *et al.*, 2015; Mostafa and Moussa, 2018; Zhu *et al.*, 2015). Restorative dentistry relies on preserving the health and function of pulp and dentin, and direct pulp capping is the procedure of treating reversible pulpal diseases by stimulating dentin bridge formation and healing exposed pulp (Li *et al.*, 2015). Calcium hydroxide has been reported to be less effective in forming dentin bridges, in the absence of which pulp tissue becomes closer to the surface and is easily invaded by oral bacteria. When the dentin bridge is less effective or absent, pulpal tissues undergo degeneration, shrinkage and atrophy; hence, newer materials were evolved in dentistry to overcome these limitations (Li *et al.*, 2015; Stanley, 1998).

2.3 Glass Ionomer Cement (GIC)

GIC is a biomaterial that has been used extensively in dentistry since 1972. The acid-base cement consists of fluoro-aluminosilicate glass Powder and polymeric acid, which is dissolved in tartaric acid and water (Mustafa *et al.*, 2020). It is regarded as a biomimetic material as it holds an identical coefficient of thermal expansion as a tooth, chemically bonds to dental hard tissue with the metal used clinically, adheres to dentin, and continuously releases fluoride (Singer *et al.*, 2023).

2.3.1 Advantages and Disadvantages of GIC

GIC has unique properties that allow it to be used widely in clinical dentistry. It was introduced in 1972 by Wilson and Kent as a cervical restorative material. They hold excellent properties along with some limitations (Almuhaiza, 2016). They are listed in Table 2.3 below.

Table 2.3 Advantages and disadvantages of GIC (Almuhaiza, 2016; Sidhu, 2011; Sidhu and Nicholson, 2016)

Advantages of GIC	**Disadvantages of GIC**
Chemically adhere to tooth structure.	They are brittle
Resistance against microleakage	Compressive strength is less.
Marginal integrity is good.	Less fracture and wear resistance
Dimensionally stable	Sensitive to moisture
biocompatible	Long term wear
Releases fluoride	The strength of conventional GIC is comparatively low.
The coefficient of thermal expansion is identical to the tooth	They cannot be subjected to heavy occlusal loading areas
Less shrinkage	
Radiopaque and translucent with good color match with tooth	

2.3.2 Clinical Uses of GIC

They are more versatile cements that can potentially be used in clinical dentistry, especially in minimally invasive procedures. The usage of GIC is listed in table 2.4.

Table 2.4 Clinical uses of GIC (Almuhaiza, 2016; Sidhu and Nicholson, 2016)

Uses of GIC
Used as liner and base
As fissure sealant
In class I and II cavities
As luting and bonding cement
Used in atraumatic restorative treatment
In class V restoration
As restorative material in primary teeth
As caries control restorative cement
For cementation of orthodontic bands and brackets
As an endodontic sealer
In endodontic root perforation and root resorption cases

Although GIC has been used for many years, the significant problems with conventional and resin-modified GIC are their brittleness and poor mechanical properties. Several attempts have been made to reinforce the material with metal, fiber, and nanoparticles to improve the strength of GIC (Nicholson *et al.*, 2020). Metal reinforcement to conventional GICs was done using a silver-tin alloy called Miracle mix and another silver-tin alloy fused with glass ionomer powder called cermet (Nicholson *et al.*, 2020). When studies were conducted to study the strength, cermet had reduced mechanical properties compared to conventional GIC (Williams *et al.*, 1992). Fibers such as carbon and alumina were used for reinforcement, and although they increased the flexural strength, the aesthetic was compromised; glass fibers were another choice to improve the properties (Nicholson *et al.*, 2020). Other than this, adding nanoparticles such as zirconia, alumina, and titanium is a newer method and has shown effective results in improving the compressive strength of GIC. Their effects depend on type, amount, and storage time (Gjorgievska *et al.*, 2020).

2.4 Mineral Trioxide Aggregate (MTA)

MTA is a biomimetic, biocompatible, hydrophilic cement. Mahmoud Torabinejad introduced it in 1993 (Singer *et al.*, 2023). It is mainly composed of Portland cement, oxides of tricalcium, bismuth, silicon, tricalcium and dicalcium silicate, and tricalcium aluminate. It can stimulate osteogenesis and cell proliferation, favors the migration and differentiation of hard tissues, stimulates dentin bridge formation, and is effective in direct pulp capping better than calcium hydroxide with superior biocompatibility, sealing ability, and marginal adaptation (Cervino *et al.*, 2020).

2.4.1 Advantages and Disadvantages of MTA

MTA has several advantages, which have made its use superior to other materials, but it has a few drawbacks. They are noted down in Table 2.5.

Table 2.5 Advantages and drawbacks of MTA

Advantages of MTA	**Disadvantages of MTA**
It has suitable antimicrobial properties.	It has a long setting time.
It provides an excellent seal.	Highly soluble
It is biocompatible and radiopaque	It shows discoloration
The less pulpal inflammatory response	Poor handling properties
The rate of formation of dentine bridge is high	The mixture has a sandy feeling and is challenging to apply and condense
The formation of a hard tissue barrier is more than calcium hydroxide	High cost
Non-resorbable and non-cytotoxic	

2.4.2 Clinical Usage of MTA

MTA is a potential calcium silicate cement favourably used in numerous endodontic procedures over other restorative materials due to its compatibility and bioactivity. Its clinical applications are tabulated in table 2.6 below.

Table 2.6 Clinical usage of MTA (Kadali *et al.*, 2020; Parirokh and Torabinejad, 2010)

Clinical uses of MTA
Choice of material in direct pulp capping
Root end filling material.
Used in vital pulp therapies
In the case of external and internal root resorption
For obturating the canals
In the apexification of immature roots
Used in root canal filling and horizontal root fractures
Sealing communication between root canal space and external root surface

2.4.3 Animal Studies on Permanent Teeth

Various studies have been conducted on permanent animal teeth to reveal the clinical success of MTA over other restorative materials in different endodontic procedures. Animal studies of MTA on permanent teeth are listed in Table 2.7.

Table 2.7 Animal studies on permanent teeth (Faraco Jr and Holland, 2001; Parirokh and Torabinejad, 2010)

Study and clinical condition	**Teeth**	**Exposure type**	**Restoration**	**Time**	**Result**
Pitt ford et al. 1996, direct pulp capping	Monkeys' teeth	Mechanical	MTA, Calcium hydroxide	5 months	MTA showed the formation of dentin bridge in all specimens and no inflammation compared to calcium hydroxide
Faraco et al. 2001, direct pulp capping	30 teeth of 3 dogs	Mechanical	MTA, Calcium hydroxide	2 months	CH displayed dentine bridge in 5 cases and inflammation in 12 cases; MTA revealed dentine bridge in all cases.
Parirokh et al. 2005, direct pulp capping	Dog's teeth	Mechanical	WMTA GMTA	2 weeks	Calcific bridge formation and no inflammation in all specimens of WMTA and GMTA
Salako et al. 2003, pulpotomy	Maxillary molars of rats	Mechanical	FC, MTA Bioactive gas, Ferric sulfate	4 weeks	MTA exhibits complete dentine formation in all specimens, an ideal pulpotomy agent
Torabinejid et al. 1995, root end filling	Dog's teeth	Caries exposure	MTA, Amalgam	10-18 weeks	MTA revealed complete cementum formation in 80% of cases
Yildrim et al. 2005, root end filling	Dog's teeth	Caries exposure	MTA, Super EBA	6 months	MTA had cementum formation in all cases, EBA showed mild inflammation and no cementum formation
Holland et al. 2007, lateral perforation	Dog's teeth	Caries exposure	MTA, Seal apex	180 days	MTA exhibited cementum formation and no inflammation in all cases; seal apex displayed inflammation even after 180 days

2.4.4 Animal Studies on Primary Teeth

MTA is a successful restorative material in many endodontic procedures in permanent teeth and was also tested in primary teeth for different procedures like pulpotomy and vital pulpal therapy. Studies conducted on primary teeth are entered in Table 2.8.

Table 2.8 Animal studies of MTA on primary teeth (Parirokh and Torabinejad, 2010; Shayegan *et al.*, 2008)

Study and clinical condition	**Teeth**	**Exposure type**	**Restoration**	**Time**	**Result**
Shayegan et al. 2008, (pulpotomy)	Pig's teeth	Carious exposure	Formocresol, Ferric sulphate, MTA, Tricalcium sulphate, WPC		Formocresol and ferric sulphate were irritating, whereas other materials were biocompatible.
Shayegan et al. 2009, (vital pulp therapy)	Pig's teeth	Carious exposure	MTA, Dycal, WPC, beta-tricalcium phosphate cement		No significant difference in any material