BACTERIAL LEAKAGE AND MARGINAL ADAPTATION OF THREE BIOCERAMICS PULP DRESSING MATERIAL

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by

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LIST OF ABBREVIATIONS

AAPD	American Academy of Pediatric Dentistry
BHI	Brain Heart Infusion
CEM	Calcium-enriched mixture
CH	Calcium Hydroxide
FPM	First permanent molar
GIC	Glass incomer cement
NaOCl	Sodium hypochlorite
MTA	Mineral trioxide aggregate
PYR	Pyrrolidonyl arylamidase
SEM	Scanning electron microscope

LIST OF APPENDICES

KEBOCORAN BAKTERIA DAN PENYESUAIAN MARGINAL (PINGGIRAN) OLEH TIGA BAHAN PEMBALUT PULPA BIOSERAMIK

ABSTRAK

Untuk menilai keupayaan pengapan dan penyesuaian marginal oleh tiga bahan pembalut pulpa bioseramik dengan menggunakan dua kaedah iaitu ujian kebocoran bakteria dan mikroskop elektron pengimbas (SEM). Lima puluh lima gigi premolar bawah pertama yang baru dicabut dibahagikan kepada lima kumpulan secara rawak; tiga kumpulan eksperimental (n=15), kumpulan kawalan positif (n=5) dan kumpulan kawalan negatif (n=5). Prosedur pulpatomi koronal yang telah diubahsuai dijalankan ke atas semua sampel kecuali sampel kumpulan kawalan negatif (n=5). Bahan pembalut pulpatomi koronal bioseramik setebal 3mm digunakan pada kumpulan 1 (Biodentine), kumpulan 2 (MTA) dan kumpulan 3 (ProRoot MTA). Bahan pambalut pulpatomi tidak digunakan pada kumpulan kawalan positif. Sampel diletakkan di dalam inkubator pada suhu 37℃ dan kelembapan 100% selama 72 jam supaya bahan set secukupnya diikuti dengan meletakkan bahan restoratif komposit. Dua lapisan pengilat kuku disapu pada semua sampel dan 3mm hujung akar dibuang. Ujian kebocoran bakteria dijalankan dengan menggunakan bakteria *Enterococcus faecalis* dan satu sampel daripada setiap kumpulan eksperimental dipotong dan diimbas dengan menggunakan SEM untuk melihat penyesuaian marginal. Data dianalisis dengan menggunakan ujian One-way ANOVA bersama dengan ujian Tukye's post-hoc. Perbezaan yang signifikan dapat dilihat pada keupayaan pengapan dan penyesuaian marginal ($p<0.05$). ProRoot MTA menunjukkan keupayaan pengapan dan penyesuaian marginal yang paling tinggiberbanding bahan yang lain. ProRoot MTA menunjukkan keupayaan pengapan dan pemyesuaian marginal yang baik berbanding kumpulan lain. Kajian ini menunjukkan ProRoot MTA merupakan pilihan bahan pembalut pulpa yang terbaik di dalam penggunaan klinikal.

BACTERIAL LEAKAGE AND MARGINAL ADAPTATION OF THREE BIOCERAMICS PULP DRESSING MATERIAL

ABSTRACT

This study aims to evaluate the sealing ability and marginal adaptation of three different bioceramics pulp dressing materials using a bacterial leakage test and scanning electron microscope (SEM). Fifty-five recently extracted lower first premolars were randomly divided into five groups, with three experimental groups (n=15), a positive control group (n=5) and a negative control group (n=5). The samples were instrumented with a modified coronal pulpotomy procedure except for the negative control group (n=5). Different types of bioceramics dressing material were placed in the cavity 3mm thickness in group 1 (Biodentine), group 2 (MTA) and group 3 (ProRoot MTA). There was no dressing material placed in the positive control group. Samples were placed in an incubator at 37℃, 100% humidity, for 24 hours to allow the material to be set, after the placement of the composite restoration. Two layers of nail varnish were applied, and the 3mm root tip was removed. The bacterial leakage test was performed using *Enterococcus faecalis*, and one sample from each experimental group was sliced and examined under SEM for marginal adaptation. Data analysis was conducted under the One-way ANOVA test, completed by Tukey's post hoc test. The groups observed a significant difference in sealing ability and marginal adaptation ($p<0.05$), wherein Biodentine showed bacterial leakage on day 6 (7%), and on day 14 (80%), MTA showed on day 9 (14%) and on day 14 (40%), ProRoot showed on day 11 (7%) and on day 14 (33%). From SEM, ProRoot MTA demonstrated the least gap between the dressing material and significantly higher penetration in dentinal tubules. ProRoot MTA demonstrated better sealing ability and marginal adaptation compared to other groups. The finding indicates that ProRoot MTA would be the best pulp dressing material for the clinical setting.

Keywords: Bacterial leakage test, bioceramics, Biodentine, MTA, ProRoot MTA, Pulpotomy, SEM.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Pulpotomy is a vital pulp therapy in which the coronal portion of the pulp is amputated and removed surgically, and the remaining radicular pulp is preserved (Solomon et al., 2015). Following the conventional procedure, a suitable wound-dressing medicament is placed on the amputation site to protect the radicular pulp against further injury and facilitate healing and repair as part of the regenerative process (Zanini et al., 2016). Depending on the coronal pulp tissue removal level, a pulpotomy can be done either partially, known as partial pulpotomy, or entirely referred to as coronal pulpotomy (Cushley et al., 2019). However, the depth of pulp removal depends upon the clinical judgment on how deeply the pulp is affected.

In Paediatric Dentistry, a coronal pulpotomy procedure can be performed either on primary molars or immature permanent teeth. Coronal pulpotomy in immature permanent teeth is gaining more attention among clinicians due to its minimally invasive treatment protocol, which is less complicated and cost-effective than any other conservative procedure, such as root canal treatment (Chen et al., 2019). Coronal pulpotomy has shown promising and improved outcomes over the decades. The coronal pulpotomy success rate is between 82.9% to 100%, which is highly related to the pulp dressing material used in the treatment (Qudeimat et al., 2017).

In the coronal pulpotomy procedure, the complete seal above the radicular pulp is from a pulp dressing material and a tooth restoration (Zanini et al., 2016). However, the healing of the radicular pulp can be compromised, and there is still a 20%-30% chance of failure (Alqaderi et al., 2016). A microbial invasion from the coronal site of

the tooth into the pulp chamber and pulp canal is the primary reason for failure in this procedure. Saliva and microorganisms from the oral cavity may rapidly migrate alongside poorly adapted coronal restorations. Additionally, the standard protocol for coronal pulpotomy procedure in immature permanent teeth may vary among dental practitioners and may also influence the final outcome. Qudeimat et al.(2017) showed in their preliminary study of a pulpotomy procedure, after placement of MTA as pulp dressing medicament, a temporary restoration of glass ionomer cement which was placed in the pulp chamber for 3 to 10 days. Another case series reported using Biodentine as a pulpotomy dressing material followed by temporary restoration, and the teeth were restored by resin composite as definitive restoration within 7 days (Poornima et al., 2017).

Various new materials have emerged as coronal pulpotomy dressing materials in today's dentistry, and each may have a different sealing ability. The bioceramics dressing materials often used as pulpal dressing after coronal pulp amputation includes mineral trioxide aggregate (MTA) and Biodentine. A study of microbial leakage carried out by (Lertmalapong et al., 2019) using various bioceramics reported different outcomes after a 5-month experimental period in which Biodentine and ProRoot MTA exhibited the best sealing ability and marginal sealing adaptation. Over the years, extensive studies on these materials used in pulpotomy procedures have examined factors such as their disinfection capability, biocompatibility, cytotoxicity, and discolouration (Aravind et al., 2022). However, limited studies assess the sealing ability of bioceramics dressing materials in coronal pulpotomy procedures.

1.2 Problem statement

The tight cervical coverage of amputated pulp with coronal sealing material could ensure a second defence against bacterial leakage besides a definitive restoration. A recent study by Sadaf (2020) proved that the most influential factor in the outcome of a coronal pulpotomy is adequate sealing of the remaining healthy pulp by bioactive material. Since the material will be applied onto the vital radicular pulp tissue on an immature permanent tooth, it must be biocompatible, not exert any toxic effects on the remaining pulp tissue, can kill, and most importantly, prevent future bacterial infection ingestion. For years, calcium hydroxide (CH) has been the gold standard dressing material (Glass and Zander, 1949, Tronstad, 1974, Ford and Roberts, 1991). It promotes healing in multiple clinical conditions and is considered the best chemical agent for promoting vital pulp tissue healing and complex tissue deposition. However, CH gets diminished over time because the material dissolves under restoration with tunnel defect, leading to bacterial leakage (Nair et al., 2011).

Various advanced bioceramics dressing materials have been proposed as an alternative to CH, such as mineral trioxide aggregate (MTA), Biodentine, and calcium-enriched mixture (Özgür et al.), providing a better sealing ability with a higher success rate. MTA is well known for its excellent sealing ability. It was reported that MTA could release sufficient calcium ions reacting with environmental phosphate and produce hydroxyapatite crystals on the surface of MTA (Asgary et al., 2009). An animal study on revascularisation showed that cemental bridges formed beneath MTA (Wang et al., 2010). The biological seal beneath the MTA may further prevent bacterial prevention. In contrast, a case report showed that coronal leakage could occur in a tooth with MTA as the canal-sealing material with a defective filling two years after revascularisation, with inflammatory cells being observed primarily on the

coronal area of the revascularized tissue (Becerra et al., 2014). A more recent study on endodontically treated teeth by Lertmalapomg et al. (2019) found a more remarkable sealing ability and marginal adaptation of ProRoot MTA and Biodentine as apical plugs compared to other bioceramics types of material using bacterial leakage test followed by scanning electron microscope (SEM).

Undoubtedly, studies on bacterial tight coronal seal material as a dressing in pulpotomy procedures are limited, and there is no previous information regarding their marginal sealing ability and its association with time, as well as bacterial ingestion into the apices. Since different materials have their advantages and disadvantages, the sealing ability and marginal adaptation need to be evaluated to recognize the most appropriate pulp dressing material for the coronal pulpotomy procedure on the immature permanent tooth.

1.3 Justification of the study

A previous study by Yavari et al. (Yavari et al., 2012) used MTA and CEM as intra-orifice barriers and performed a bacterial leakage test to check the sealing ability of these materials with different types of final restoration. The CEM and MTA groups showed superior sealing ability when restored with a composite restoration. In a more recent study by Lertmalapong et al. (2019), ProRootMTA demonstrated excellent sealing ability and marginal adaptation ability compared to other bioceramics apical plugs. These studies primarily assessed the marginal sealing ability using different bioceramics as an apical plug. Our study compares the most used bioceramics dressing material for coronal pulpotomy procedures in paediatric dentistry and assesses their sealing ability by focusing on the coronal side of the tooth. Moreover, most studies on

dressing material for coronal pulpotomy mainly focus on biocompatibility, such as cytotoxicity (Kang et al., 2021).

Furthermore, this *in vitro* study investigates the bacterial leakage and coronal marginal adaptation of bioceramics as dressing material in coronal pulpotomy procedures. This procedure's effectiveness depends on the dressing material's marginal sealing ability. In addition, the gap between the material dressing and dentinal walls can cause bacterial leakage to the radicular pulp. As a result, the healing of the vital radicular pulp is compromised. The expected outcome of this *in vitro* study will help clinicians identify the best material pulp dressing for coronal pulpotomy procedures. This is the first *in vitro* study to investigate bacterial leakage on coronal pulpotomy using the modified bacterial leakage model. The evaluation of marginal adaptation between the dressing material and tooth structure will be carried out further by an SEM (Scanning electron microscope) to support the findings.

1.4 Research question

1) Is there any significant difference in the sealing ability and overall survival leakage time of different bioceramics pulp dressing materials for coronal pulpotomy?
2) Is there any significant difference in the adaptability of different types of bioceramics dressing material for coronal pulpotomy?

1.5 Null Hypotheses

1) There is no significant difference in the sealing ability and overall survival leakage time of the different types of bioceramics pulp dressing material for the coronal pulpotomy.
2) There is no significant difference in the adaptability of different bioceramics pulp dressing material types for the coronal pulpotomy.

1.6 Objectives

1.6.1 General Objective

To evaluate bacterial leakage and marginal adaptation of bioceramics types of pulp dressing material for coronal pulpotomy.

1.6.2 Specific Objectives

1) To determine the sealing ability of three types of bioceramics pulp dressing materials using a bacterial microleakage test.

2) To determine and compare the overall survival leakage time of three types of bioceramics pulp dressing materials using a bacterial microleakage test.

3) To compare the marginal adaptation of three types of bioceramics materials as pulpotomy dressing to the dentine walls under the scanning electron microscope (SEM).

CHAPTER 2

LITERATURE REVIEW

2.1 Pulpotomy

Vital pulp therapies are mostly recommended for primary teeth with deep or extensive carious lesions with reversible pulpitis (Dhar et al., 2017). If dental caries is left untreated, it could result in dental pulp inflammation, severe pain, and necrosis followed by abscess formation (Cushley et al., 2019). Vital pulp therapies like pulpotomy in primary teeth are a widespread treatment protocol due to a high success rate and quality of evidence compared to other essential pulp therapies (Dhar et al., 2017). Pulpotomy is usually advised in teeth with histological inflammation of the coronal pulp. The aim is to remove inflamed coronal pulp tissue either partially or wholly to restore healthy radicular pulp (Igna, 2021). Factors concerning the success rate of pulpotomy include accurate diagnosis before treatment (Waterhouse et al., 2011), well-handled isolation, thorough disinfection (Rutherford and Gu, 2000), rigorous restoration using glass ionomer cement (GIC) and resin or amalgam and different pulpotomy-dressing agents. Lin et al. (2021) reported in their umbrella review on pulpotomy, based on an online database and five textbooks from 1970 to 2021, that both coronal and partial pulpotomy have a high rate of success from 88.5 % to 90.6%. In a study by Wang et al. (2017), 375 teeth were retreated by both types of pulpotomies (partial and coronal) and direct pulp capping, and direct pulp capping shows frequent pulp necrosis and infection after treatment. On the other hand, pulpotomy shows a satisfactory pulp survival rate.

2.2 Partial pulpotomy

Partial pulpotomy is a widely suggested treatment protocol for teeth that are asymptomatic and have 1 to 2 mm carious exposure. Moreover, according to American Academy of Pediatric Dentistry (AAPD) (2020), bleeding can be stopped within 2 minutes in the teeth. Previous research has determined that pulp is stable if haemostasis can be achieved in less than 5 minutes using a haemostatic agent such as sodium hypochlorite (NaOCL) or sterile saline (Özgür et al., 2017). Partial pulpotomy is a potential treatment method for carious exposed teeth to avoid or delay root canal treatment (George, 2020). Eggmann et al. (2022) took 111 cases for their study in 18-85 years old patients. After treatment, the success rate was 98.4% for tooth survival and 89.1% in maintaining the pulp's vitality. Partial pulpotomy was not recommended in the case of profuse bleeding pulp that was difficult to handle. Compared to moderately or poorly bleeding pulp bleeding, excessive pulp bleeding indicates a lower likelihood of success (Özgür et al., 2017).

2.3 Coronal pulpotomy

Recently, most clinicians preferred performing coronal pulpotomy in questionable pulp status, especially in young, immature permanent teeth, rather than conventional root canal treatment. Coronal pulpotomy or full pulpotomy, or cervical pulpotomy, is a successful technique in both primary and permanent teeth in children and adults (Lin et al., 2021, Taylor et al., 2020). The coronal pulpotomy removes the entire coronal pulp tissue up to the root orifice level. There are a few factors concerning the success rate of coronal pulpotomy: accurate diagnosis before treatment, well-handled isolation, disinfection, rigorous restoration using glass ionomer cement (GIC) and resin or amalgam and different pulpotomy-dressing agents. In addition, clinicians

mostly rely on clinical appearances, such as the colour and occurrence of bleeding, to assess the pulp condition (Deshmukh et al., 2018). However, the most influential factor in the outcome of a coronal pulpotomy is adequate sealing by a bioactive material (Sadaf, 2020). Alqaderi et al. (2016) proved that coronal pulpotomy with MTA as dressing material has a high success rate of 90% and can be considered a successful alternative to root canal treatment for caries exposed to immature first permanent molar teeth (FPM) in children. Another recent study also showed that immature FPM indicative of irreversible pulpitis has a success rate following coronal pulpotomy with MTA (Qudeimat et al., 2017). In several cases, patients cannot undergo root canal treatment because of the cost, and without insurance, they have no choice other than to extract the teeth. Coronal pulpotomy is cost-effective, less invasive, less time-consuming and uncomplicated for both patient and dentist. Meta-analysis and systemic review are considered the highest level of evidence. All systematic reviews on coronal pulpotomy showed a high success rate compared to root canal therapy. Coronal pulpotomy is a safe and evidence-based procedure that can offer treatment for immature permanent teeth in case of irreversible pulpitis (Sadaf, 2020).

However, evidence has shown that in 6 to 18-year-old patients, root canal treatment was only being performed in approximately only 20% of teeth with signs and symptoms indicative of irreversible pulpitis, whereas 24% and 59% of teeth were extracted or received temporary restorative treatments, respectively (Al-Madi et al., 2018). Asgary et al. (2015) demonstrated in their study that root canal treatment is contrary because it is time-consuming, expensive, and a complicated procedure. The coronal pulpotomy or complete pulpotomy procedure is an option with comparable success to root canal treatment but without interfering with the vitality of the infected tooth.

A coronal pulpotomy is also considered a retreatment option for partial pulpotomy (Wang et al., 2017). Asgary et al. (2015, 2014) described their randomised controlled trial comparison between root canal treatment and coronal pulpotomy with irreversible pulpitis and long-term radiographic follow-up, up to 60 months post-treatment. On the 12-month follow-up, the radiographic success rate of root canal treatment was 89%, and coronal pulpotomy was 92.2%. On the other hand, on the 60-month follow-up, the root canal treatment success rate was 65.8%, and coronal pulpotomy was 71.3%. Cushley et al. (2019) evaluated the success rate of coronal pulpotomy in their systemic review. During the 12-month follow-up, the radiographic success rate was 95.45%, and the clinical success rate was 97.4%.

2.3.1 Indications for a coronal pulpotomy treatment (Baik et al., 2018)

1) The pulp is inflamed to the deeper levels of the coronal pulp.

2) Traumatic exposure to a pulp for more than 72 hours.

3) Carious exposure to primary, young permanent, and mature teeth.

2.3.2 Contraindication of coronal pulpotomy treatment (Baik et al., 2018)

1) Evidence of pulp necrosis.

2) Root resorption is more than two-thirds.

3) Spontaneous discomfort of the tooth.

4) A non-restorable tooth.

2.3.3 Clinical significance of coronal pulpotomy (Alqaderi et al., 2016)

1) Intermittent treatment option for treatment of curiously exposed vital pulp.

2) Economical treatment option for the patient.

2.3.4 Investigation for coronal pulpotomy (Solomon et al., 2015)

1) Clinical evaluation:

   The clinician will evaluate by taking proper history, performing an intraoral and extraoral examination, and thermal and pulp testing.

2) Radiographic evaluation:

   A radiograph is taken to confirm the demineralization level of teeth and to exclude teeth with other complications.

2.4 Coronal pulpotomy procedure on immature permanent teeth

The aims of the coronal pulpotomy procedure in immature permanent teeth are to prevent pre-operative contamination, control pre-operative infection and achieve a complete seal above the healthy radicular pulp area. For each clinical case, caries is removed carefully using a round bur with a high-speed handpiece. After the pulp exposure, the entire coronal pulp is removed from the pulp chamber to the pulpal floor (Solomon et al., 2015). This procedure removes the coronal pulp tissue to eliminate the infected or contaminated pulp, leaving the healthy vital radicular pulp intact. The pulp amputation level should be chosen carefully because inflamed pulp may also enter the canal orifice. Not taking an appropriate decision at the time of treatment may lead to treatment failure due to remaining inflamed pulp tissue (Berman and Hargreaves, 2020). After pulp tissue removal, haemostasis is obtained by using a moist

cotton pellet pressure and copious irrigation. Sterile solution and sodium hypochlorite are the most popular rinse solutions used in irrigation and haemostasis stages. When comparing sterile solution to sodium hypochlorite, sodium hypochlorite has been one of the most widely used disinfectants for root canal treatment for many years. It is now used as a haemostatic agent due to its ability to control bleeding while disinfecting the cavity. When employed against pulp tissue, a concentration of 1.5 per cent to 6 per cent sodium hypochlorite appears to be the most effective, affordable, and safe to use, as this concentration had no detrimental effects on pulp cell recruitment, cytodifferentiation or reparative dentine deposition (Chinadet et al., 2019). Furthermore, sodium hypochlorite has been recommended as a diagnostic tool for determining pulpal inflammation; bleeding that can be controlled in less than 10 minutes has a favourable outcome or prognosis (Chinadet et al., 2019).

After haemostasis is achieved, the amputation site is gently filled with an approximate 3mm pulpotomy dressing agent (Tran et al., 2021) is applied to the remaining pulp, allowing it to heal (Solomon et al., 2015). An ideal medicament should be non-toxic, biocompatible, have antimicrobial and anti-inflammatory activity, induce mineralisation, and establish a tight seal (Özgür et al., 2017). In addition, it should leave radicular pulp vital, healthy, and enclosed within the odontoblastic-lined-dentine chamber and compatibility between pulp and surrounding tissue physiology (Chandrashekhar and Shashidhar, 2014).

Most of the previous studies employed Fuji IX Glass Ionomer Cement as a base material before filling the cavity with a temporary or permanent restoration. Glass Ionomer Cement (GIC) can resist bacterial invasion and microleakage, as well as the ability to bind chemically with the tooth structure, preventing potentially hazardous substances from penetrating the pulp. Even though MTA has been found to have a

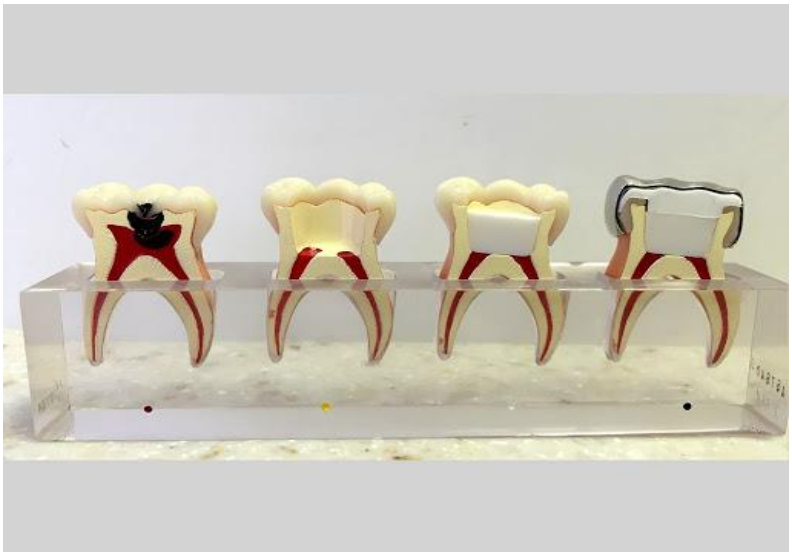
stronger sealing ability than CH, it has shown limited or no adherence to dentine, hence both materials (MTA and CH) were coated with light cure glass ionomer cement to remove any possibility of failure (Özgür et al., 2017). Apart from GIC, Chinadet et al. (2019) used Biodentine altogether for pulp dressing, base, and interim restoration, and the result showed a good outcome. Thereby, utilising glass ionomer cement or another base material did not affect the treatment's outcome. However, more research is needed to confirm the findings.

Finally, definitive restoration is placed to ensure the functionality of the affected tooth. Teeth were commonly restored with resin-modified glass ionomer cement (RMGIC) and bulk-fill composite, conservative glass ionomer cement or solely composite (Rechithra et al., 2021). Most authors considered that a well-sealed coronal restoration is more important than the dressing material used in vital pulp therapy for the long-term preservation of vitality and function of the teeth. The ability of pulpal treatment to work depends on bacterial penetration through the leakage.

Coronal leakage is one of the most common reasons for healing failure. Therefore, an excellent coronal seal should be obtained to avoid bacterial recontamination and ensure a favourable outcome.

According to Barthel et al. (1999), they were placing the definitive restoration during the first two days after the exposure had a substantial impact on the success rate of pulp capping of carious exposure in permanent teeth. Another point of view, Elmsmari et al. (2019) mentioned that some studies preferred to perform definitive restoration in the same appointment to avoid the possibility of microleakage. However, in a study by Qudeimat et al. (2017), failure of three cases of pulpotomies was reported when the final restoration was placed immediately after the procedure.

Stainless steel crown (SCC) has been suggested as superior restorative material because of its outstanding sealing qualities, high compressive strength, and appropriate retention compared to standard restoration. According to Qzgur et al. (2017), MTA and final restoration with SCC are the critical factors for the greater success rate seen in the research. On the other hand, composite resin, the most common coloured restorative material used, had also shown a favourable result, and two of the studies used amalgam as the final restoration. A long-term radiologic study conducted by Mass et al. (2011) mentioned the failure of 3 studies, 2 of which were restored with amalgam and 1 with composite. However, it does not influence the overall outcome of the treatment. Thereby, the choice of final restorative material does not directly affect the success of the treatment as long as an excellent coronal seal can be achieved.



A B C D

Figure 2.1 Coronal pulpotomy procedure illustrated permanent lower molar (A-Infected Tooth, B- Removal of Coronal Pulp Tissue, C- Placement of Dressing Material, D- Placement of Coronal Restoration) (https://www.odontovida.com/2020/06/what-is-pulp-therapy-what-are-pulp.html)

2.5 Historical progress of dressing material for pulpotomy

An ideal pulpotomy dressing material should be biocompatible, capable of complex tissue formation, have disinfectant properties, excellent sealing ability, and lack cytotoxicity (Aravind et al., 2022). This is important as the pulp-dentine complex healing potential, or radicular pulp, depends on this. The selection of dressing material can influence the success rate of vital-pulp therapy (Manzoor et al., 2021). Several studies have also shown a strong relationship between the dressing material used with different outcomes of pulpotomy. Mineral trioxide aggregate (MTA), Biodentine, Formocresol, ferric sulphate, glutaraldehyde, CH, Er, Cr: YSGG, EMD, and calcium-enriched cement (Bossù et al., 2020).

2.6 Bioceramics

Biomaterial with ceramic base that are designed to be used in a medical device or as a medical device that are exposed to proteins, cells, tissues, organs and organ systems. The types of ceramics used in the field of biomedicine are called bioceramics. Bioceramics are non-metallic, inorganic and biocompatible which their mechanical properties are similar to the hard tissue they are intended to replace or repair. They are non-corrosive, chemically stable, and interact well with the organic tissue (Davaie S. et al, 2021).

For many years, bioceramics have proved to be successful in the medical field. For example, they are widely used as joint replacement in orthopedic surgery . They also have many applications in dentistry including orthodontic brackets, endodontic sealers, prosthodontic devices, restorations, and repairing maxillofacial and periodontal defects. The logic for the extensive use of ceramics in dentistry and biomedicine is due to their inertness compared to metals. During the past two decades, interest has shifted towards bioceramic materials which can induce physiological function as well as being able to form a close bond with hard tissues(Davaie S. et al, 2021).

2.7 Material for partial pulpotomy

To assess the success of coronal pulpotomy, the standard clinical criteria are symptom-free, absence of radiographic abnormality and continued root formation. In 2020 Ramanandvignesh et al. (2020) performed an *in vivo* study using MTA, Biodentine and Er, Cr: YSGG as a coronal pulpotomy material. Clinically and radiographically, the overall success rate of the three pulpotomy groups was 85.5%, with no statistical difference among the groups for the observation period of 3, 6 and 9 months. Even though Biodentine turned out to be the most successful material, another study by Caruso et al. (2018) used CH, Formocresol and MTA as dressing material on 360 paediatric patients and 400 teeth. After 30 months of follow-up, the clinical success rates were 100% for MTA, 95.2% for Formocresol, 96.4% for ferric sulfate, and 85% for CH.

2.8 Material and medicament dressing in Coronal Pulpotomy

Recently, various dressing materials in pulp management have been introduced. These materials each have their own recommended concentration and potentially bioactive ingredients, leading to a claim of additional healing benefits.

The ideal criteria for a pulpotomy medicament are described in Table 2.1 (Azmi et al., 2021).

Table 2.1 Pulpotomy medicament

Criteria	Details
Biocompatible	Should not exert toxic effects on the vital pulp cells
Wide range of antibacterial effects	Able to kill all the bacteria that cause the infection
Good healing property	Promotes healing of the radicular pulp
Readily absorbable	Does not interfere with physiological root resorption
Good haemostatic effect	Able to arrest the bleeding from the pulp tissue
Good sealing ability	Able to seal off and protect the underlying pulp tissue from future infection
Has anaesthetic effects	Able to numb the pulp tissue without the need for dental injection
Cheap	Reduce the cost of the treatment
Does not cause tooth discolouration	For aesthetic reasons, mainly if used in anterior teeth
Easy to handle and not technique sensitive	The manipulation of the material/medicament is easy and does not require extra instrumentation
Hydrophilic	Does not disintegrate in the presence of moisture and blood
Has anti-inflammatory	Able to reduce the pulp inflammation caused by dental caries

As more pulpotomy dressing agents are introduced in treating pulp exposure caused by caries or trauma, different outcomes of each treatment should be evaluated. CH and MTA are among the commonly used medicament for coronal pulpotomy. More bioactive materials of pulpotomy-dressing agents intrigued clinicians, such as CEM (calcium-enriched cement) and modified calcium silicate-based materials (MTA, Biodentine) with the claim of additional benefits of one material over another.

Herman introduced CH into the dental world in the 1920s. It has been widely used as pulpotomy dressing material due to its antibacterial property and the ability of the material to induce calcific barrier formation. It comes in the form of a paste, which is highly alkaline, with a pH between 11 and 12. The material will dissociate into calcium and hydroxyl ions in an aqueous environment. These hydroxyl ions are very potent that could elicit damage to the bacterial cytoplasmic membrane and inactivate the bacterial enzymes. At the same time, through the activation of the alkaline phosphatase enzyme, the hydroxyl ions also provide an alkaline environment, favouring the reparative process and active calcification (Jahromi and Motamedi, 2019). However, the success rate of pulpotomy with non-setting CH was only 64% (Liu et al., 2011). This is mainly due to the tunnelling defect present within the calcific barrier that was formed. (Morotomi et al., 2019). The formation of the calcific barrier is a slow process in which, during the process, some collagen bundles and blood vessels might be trapped and calcify within the barrier itself, creating a tunnel. The presence of this tunnel will lead to microleakage of the barrier and subsequent treatment failure. Apart from that, the long-term usage of non-setting CH also causes desiccation of the dentinal protein and collapse of the collagen framework, which predisposes the tooth towards internal resorption and root fracture (Schroder, 1973, Seltzer and Bender, 1975). Silva et al. (2019) compared CH and MTA as pulpotomy

material, where CH with the saline group in 3 to 12-month follow-up showed internal resorption up to 67%, inner reticular bone resorption and furcation radiolucency up to 36%. Meanwhile, calcium with polyethene glycol group showed reticular bone resorption and furcation radiolucency of 9% and internal resorption of 18% in 3 to 12-month follow-up as well.

MTA has a pH of 12.5, which is similar to CH (12.5-12.8). This high alkalinity contributes to its antibacterial activity against *Streptococcus mutans* and *Lactobacilli*, the main bacteria that cause dental caries (Maria de Lourdes et al., 2008). Unlike CH, MTA has an exceptional sealing ability, and a rigid tissue barrier is formed to provide a tight seal against bacterial leakage. This is one of the reasons for the superiority of MTA compared to other materials used for pulpotomy. A previous study showed that good sealing of MTA as a root-end filling material was achieved with a thickness of around 4 mm (Lertmalapong et al., 2019)

ProRoot MTA is an excellent material for root repair and has been used successfully for years. Recently, the FDA cleared this material to be used in paediatric pulpotomy cases. A recent randomized controlled trial showed that ProRoot MTA exhibited an overall success rate of 92% for partial pulpotomy in permanent teeth of 6 to 18-year-old patients with signs and symptoms indicative of irreversible pulpitis (Uesrichai et al., 2019). When gently placed over the exposed side, ProRoot MTA can create a biocompatible seal that is suitable for replacing pulp in the pulp chamber to prevent infection. ProRootMTA can decrease bacterial migration significantly. ProRootMTA has an average setting time and can set in the presence of moisture. In several studies, ProRootMTA has shown less dye leakage and remarkable marginal adaptation than other materials (Hashem and Hassanien, 2008, Kubo et al., 2005). Maltezos et al. (2006) reported that ProRootMTA exhibited the best sealing ability in

a bacterial leakage test. A recent *in vitro* study also showed that ProRoot MTA has less leakage than Endoseal MTA material (Dastorani et al., 2021).

Neo MTA Plus is commonly used in paediatric pulp therapy due to its cost-effectiveness. This is a pure MTA and does not contain resin as opposed to ProRoot MTA. The purpose of incorporating resin in the MTA mix was to improve material flow dentine bonding and set time, and reduce micro-leakage (Alsanouni and Bawazir, 2019). Karobari et al. (2021) performed a dye leakage test in their study using Glass ionomer cement, MTA, Biodentine, ProRootMTA and Neo MTA plus. This study showed that Neo MTA plus had a minimal dye depth.

MTA Fillapex is a newly launched material. Despite being a newly-launched material, it has proven to be an excellent choice for coronal pulpotomy because of the biological properties of MTA. MTA Fillapex has adequate radiopacity, alkaline pH, and high flowability, and its sealing ability is remarkable. However, MTA Fillapex is a technique-sensitive material with less working time, low compressive strength, and high cost. The solubility of MTA Fillapex is high, which can cause bacterial leakage in coronal pulpotomy. Several studies showed an excellent outcome when MTA Fillapex comes in contact with vital pulp (Vitti et al., 2013).

Biodentine is a new bioactive calcium silicate-based cement developed using the MTA-based cement technology, with some improvement in the physical strength and material handling. Biodentine has similar indications and mode of action to CH, with the aim to induce pulp tissue repair through the formation of the hard tissue barrier to wall off the bacterial infection (Rajasekharan et al., 2014). However, the setting time for Biodentine is faster than MTA, mainly due to the presence of calcium carbonate, which acts as an accelerator. The mixing of Biodentine also requires less

water than MTA, which contributes to the material's high compressive strength with low porosity and microleakage (Kaur et al., 2017, Grech et al., 2013). Biodentine showed less bacterial leakage than other bioactive materials (Revathi Bashyam et al., 2021). A similar *in vitro* study by Sadana et al. (2018) found that Biodentine performed remarkably in a microleakage test and had better marginal adaptation than MTA.

Although Bioceramics has a high success rate as a coronal pulpotomy dressing material, the success rate may differ in the long-term follow-up. A recent study (Kang et al., 2021) showed that the failure rate had increased from 4% to 6.6%, depending on the dressing material in a four-year follow-up of different pulpotomy dressing materials. This delayed failure is usually caused due to the absence of hard tissue formation or newly formed hard tissue failing to act as a protective barrier against bacterial leakage (Elmsmari et al., 2019). It can be assumed that bacterial leakage could play a role in pulp necrosis and periapical involvement in a more extended follow-up period.

2.9 Microleakage Testing

Microleakage is the clinically undetectable passage of fluids, bacteria, ions or molecules between a tooth and the filling or restorative material. Alternatively, microleakage is the diffusion of oral fluids, bacteria, molecules or ions into the tooth and restorative material interface (Muliyar et al., 2014). The concept of microleakage affecting treatment outcomes has been known for over a hundred years (American Association of Endodontics, 2002).

Microleakage typically occurs in two-level: micron-level leakage, also known as bacterial leakage and submicron-level leakage, which is called nano leakage (Muliyar et al., 2014).

During bacterial leakage, cariogenic bacteria get access to the tooth through the margin of restoration and are capable of causing a successful proliferation along with the area, resulting in recurrent caries. However, the marginal gap size between restoration and tooth is still questionable (Muliyar et al., 2014). With the marginal gap, the recurrent rate of caries and the risk of bacterial leakage increase. However, the bacteria found in the tooth or restorative material interference or its origin is still uncertain, and their contribution to recurrent caries still needs to be established. However, it is said that bacteria trapped in the smear layer can multiply and cause microleakage (Muliyar et al., 2014).

Nano leakage is a situation where the gap between restoration and tooth permits molecules and ions to gain access and courses microleakage at the nano level. The passage of fluid through dentin has been reported to be affected by dentin permeability that is markedly influenced by several factors, including volume of dentinal tubules, dentin smear layer, dentin calcification and topical applications (Muliyar et al., 2014).

The cause of microleakage is broadly divided into two categories:

1. Apical Leakage

Apical leakage is the most common factor for endodontic failure. Apical leakage depends upon many factors, such as the chemical and physical properties of root canal filling materials, different filling techniques and the absence and presence of a smear layer (Muliyar et al., 2014).

2. Coronal Leakage

Coronal leakage is influenced by many variables, such as contact between the oral bacterial flora and root canal tubule inlets. Coronal leakage is the most common cause of loss of temporary restoration and inadequate permanent restoration in the crown (Muliyar et al., 2014).